(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 November 2007 (29.11.2007)

PCT

(10) International Publication Number
WO 2007/136819 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2007/012048

(22) International Filing Date: 18 May 2007 (18.05.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/747,767 19 May 2006 (19.05.2006) US

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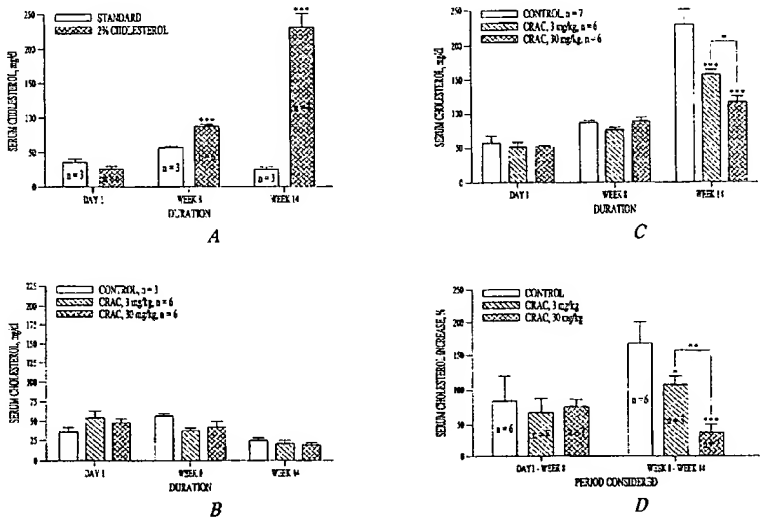
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF A PEPTIDE CONTAINING A CHOLESTEROL INTERACTION SEQUENCE



(57) Abstract: Methods of using a molecule having a cholesterol recognition/interaction amino acid sequence such as a cholesterol recognition/interaction amino acid consensus sequence are provided.

WO 2007/136819 A2

USE OF A PEPTIDE CONTAINING A CHOLESTEROL INTERACTION SEQUENCE

Related Application

This patent application claims the priority benefit of U.S. Provisional Patent Application Serial No. 60/747,767 filed May 19, 2006, which application is incorporated herein by reference.

Background

Coronary heart disease (CHD) is an important cause of mortality and morbidity in western countries. The pathophysiology of CHD is multifactorial and complex. High serum levels of cholesterol are associated with atherosclerosis and an increased risk of CHD. Detailed studies have shown that high LDL cholesterol and/or low HDL cholesterol in serum are major risk factors to develop CHD, although the very low density cholesterol (VLDL) precursor of LDL is also considered to play a significant role in the development of CHD (NECP-ATP III). Various clinical trials have demonstrated the usefulness of decreased LDL in reducing the risk to develop CHD and ameliorate the outcome of the disease. Over the years, numerous compounds have been developed to control hypercholesterolemia. Among the most widely used hypolipidemic drugs are fibrates and statins.

Fibrates, such as bezafibrate and clofibrate, lower progression of atherosclerotic lesions mainly through activation of the nuclear receptor peroxisome-proliferator activated receptor-alpha (Corton et al., 2000; Desvergne and Wahli, 1999; Robillard et al., 2005). Activation of this receptor induces the expression of genes involved in fatty acid beta oxidation as well as lipoprotein lipase and apolipoprotein A-I and A-II gene expression (Corton et al., 2000; Desvergne and Wahli, 1999; Robillard et al., 2005). Activation of peroxisome-proliferator activated receptor-alpha also leads to hepatocarcinogenesis (Reddy and Azanoff, 1980; Corton et al., 2000) and toxic myopathies, which have been reported to be related to the administration of fibrates (Galiana et al., 1995).

This raises questions about the use of fibrates in the clinic, although there is limited data to support hepatotoxicity in humans.

Hypocholesterolemic drug inhibitors of the hydroxymethylglutaryl-CoA reductase (statins) have been proven to be extremely efficient in lowering LDL levels in hypercholesterolemic patients as well as in CHD prevalence. However, although remarkably efficient, statins are hampered by major issues. Targeting the key enzyme of cholesterol synthesis also alters the synthesis of ubiquinone, a major constituent of the mitochondria respiratory chain. This effect is believed to be responsible for rhabdomyolysis, a side-effect that could occur during treatment with statins. Moreover, lipophilic statins penetrate the brain where they inhibit cholesterol synthesis required for neurogenesis, thus raising questions on the safety of long-term treatment with lipophilic HMG-CoA reductase inhibitors. Finally, the effect of statins on HDL cholesterol level is limited compared to what might be required for an effective therapy aiming at increasing HDL cholesterol rather than decreasing LDL cholesterol.

In steroid synthesizing tissues, cholesterol is the precursor of the steroids formed. Steroidogenesis begins with the transport of cholesterol from intracellular sources into mitochondria (Jefcoate, 2002), a process mediated by the peripheral-type benzodiazepine receptor (PBR). PBR is a high affinity cholesterol and drug binding protein located in the outer mitochondrial membrane (Papadopoulos et al., 1990; Lacapere and Papadopoulos, 2003). An 8-11 amino acid domain in the carboxy-terminus of PBR was identified and characterized as the cholesterol recognition/interaction amino acid consensus (CRAC) (Li and Papadopoulos, 1998; Li et al., 2001; Jamin et al., 2005). Since the cholesterol-binding domain of PBR, CRAC (Li and Papadopoulos, 1998), was identified, this motif has been used to predict proteins that bind cholesterol and will partition into cholesterol-rich regions of a membrane (Epand et al. 2005a). Indeed, the viral accessory protein Nef was shown to contain a CRAC domain and transport newly synthesized cholesterol to the site of viral budding (Zheng et al., 2003). The HIV-1 transmembrane protein gp41 was also shown to interact with cholesterol at a CRAC domain (Vincent et al., 2002, Epand et al., 2005b).

Therefore, there is a need for drugs that do not target an enzymatic pathway or do not induce global changes of gene expression, but instead directly target cholesterol present in the blood.

Summary of the Invention

The invention provides therapeutic methods which employ a cholesterol recognition/interaction amino acid sequence such as a cholesterol recognition/interaction amino acid consensus (CRAC) sequence as a hypocholesterolemic agent. As described hereinbelow, the use of an 8 amino acid CRAC sequence (VLNYYVWR; SEQ ID NO:1) found in a peripheral-type benzodiazepine receptor (PBR) lowered cholesterol in two different hypercholesterolemic animal models, the ApoE knock-out B6.129P2-Apoetm1Unc/J mice and the 2% cholesterol diet fed guinea pigs, and had an impact on the development or progression of atherosclerosis. The CRAC sequence may be employed to prevent, inhibit or treat a variety of disorders including but not limited to hypercholesterolemia, low HDL levels, viral infection, obesity, diabetes, glucose intolerance, atherosclerosis, gallstones, and other disorders associated with unregulated or aberrant accumulation of cholesterol or a failure to maintain cholesterol homeostasis.

The invention thus provides a method to prevent, inhibit or treat hypercholesterolemia. The methods include administering to a mammal in need thereof a composition having a cholesterol recognition/interaction amino acid consensus sequence having formula (I):

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid, or a composition having an isolated peptide having a cholesterol recognition/interaction amino acid sequence having formula (II): U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues. If the composition includes a peptide, e.g., a sequence that is less than that found in a naturally occurring polypeptide, the peptide may have sequences found in the corresponding naturally occurring polypeptide or may have substantially the same sequence (substantially identical), for instance, one with one or more

substitutions. Such a peptide may be prepared by recombinant or chemical synthesis. For instance, a peptide useful in the invention may have a sequence that is 20% or less, e.g., 15%, 10%, 5% or 1% or less, than a corresponding full length polypeptide but is preferably at least three amino acids in length. A peptide useful in the invention thus has 3 to about 50, or any integer in between, e.g., 5, 8, 10, 15, 20, 25, 30 or 35, amino acid residues, and may be part of a larger synthetic or isolated complex. In one embodiment, the peptide is part of a fusion polypeptide, where the fusion partner may be a full-length heterologous protein or hetoerlogous peptide. In one embodiment, the peptide may include additional sequences corresponding to those which are N-terminal, C-terminal, or both, to a naturally occurring cholesterol recognition/interaction amino acid sequence. For instance, ATVLNYYVWRDNSGRRGGSRLPE (SEQ ID NO:25) corresponds to a wild-type PBR sequence having a cholesterol recognition/interaction amino acid sequence.

Also provided is a method for reducing serum cholesterol in a mammal. The method includes administering to a mammal a composition having cholesterol recognition/interaction amino acid consensus sequence having formula (I)

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid, or a composition having an isolated peptide having a cholesterol recognition/interaction amino acid sequence having formula (II) U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues, in an amount effective to reduce serum free cholesterol and bile total cholesterol.

Further provided is a method for increasing HDL levels in a mammal. The method includes administering to a mammal a composition having cholesterol recognition/interaction amino acid consensus sequence having having formula (I)

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid, or a composition having an isolated peptide having a cholesterol recognition/interaction amino acid

sequence having formula (II) U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues, in an amount effective to increase HDL levels.

The invention also provides a method to prevent, inhibit or treat viral infection. The method includes administering to a mammal an effective amount of a composition comprising a cholesterol recognition/interaction amino acid consensus sequence comprising having formula (I)

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid, or a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence having formula (II) U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues. In one embodiment, the infection is a lentiviral infection, e.g., HIV infection.

Also provided is a method to prevent, inhibit or treat obesity. The method includes administering to a mammal an effective amount of a composition comprising a cholesterol recognition/interaction amino acid consensus sequence having formula (I)

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid, or a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence having formula (II) U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

Further provided is a method to prevent, inhibit or treat diabetes or glucose intolerance. The method includes administering to a mammal an effective amount of a composition comprising a cholesterol recognition/interaction amino acid consensus sequence having formula (I)

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid, or a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence having formula (II) U-L-N-Y-X, wherein U is absent or 1 to 6 amino

acid residues, and wherein X is absent or 1 to 7 amino acid residues.

Thus, the invention provides compounds for use in medical therapy, such as peptide-based agents that lower cholesterol, optionally in conjunction with other compounds. Accordingly, the compounds of the invention are useful to inhibit or treat hypercholesterolemia, reduce serum cholesterol, increase HDL levels, prevent or treat viral infection, inhibit or treat obesity, or inhibit or treat diabetes or glucose intolerance. Also provided is the use of the peptide-based agents for the manufacture of a medicament to inhibit or treat hypercholesterolemia, reduce serum cholesterol, increase HDL levels, prevent or treat viral infection, inhibit or treat obesity, or inhibit or treat diabetes or glucose intolerance.

Brief Description of the Figures

Figures 1A-1D. Effect of increasing concentrations of wild-type and mutant CRAC containing PBR-CTF on apoE and apoAI-cholesterol binding examined by a cholesterol protein binding blot assay (CPBBA). 2 μg of human apoE (A, B) or apoAI (C,D) were incubated with ^{3}H-cholesterol (0.1 μCi for apoE, 0.02 μCi for apoAI) in the presence or absence of increasing concentrations of wild-type (A,C; NH_2-ATVLNYYVWRDNSGRRGGSRLPE; SEQ ID NO:25) and mutant (B,D; NH_2-ATGLNSSVWLDNSGRRGGSRLPE: SEQ ID NO:26) CRAC containing PBR-CTF in 20 μL volume at 37°C for 1 hour (for apoE) or 2 hours (for ApoAI). CPBBA and image analysis were performed. Results shown are representative of 3 independent experiments.

Figure 2. Effect of CRAC treatment on hypercholesterolemic ApoE knock-out B6.129P2-Apoetm1Unc/J mice. ApoE knock-out B6.129P2-Apoetm1Unc/J mice weighing 18-22 grams at study initiation received one daily intraperitoneal (i.p.) injection of CRAC solution at 10, 30 and 100 mg/kg (0.15 mL/kg) or vehicle for 14 days. At the end of the treatment period, blood was withdrawn by cardiac puncture and total cholesterol measured. Data shown are means ± SD (n = 11-12 as indicated in the figure). Results were analyzed by one way ANOVA.

Figures 3A-3D. Effect of CRAC treatment on serum total cholesterol levels in guinea pigs fed with a high cholesterol diet. Hartley male guinea pigs

were fed with standard or 2% cholesterol enriched diet for 14 weeks. CRAC treatment started at the end of week 8 until the end of the experiment. Guinea pig received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 mL/kg) or vehicle every other day. Blood was withdrawn under anesthesia at the study initiation, at the end of week 8 and week 14 for total cholesterol measurement. A) Evolution of total cholesterol concentration measured at day 1, week 8 and week 14 in guinea pigs fed with standard- or 2% cholesterol diet. B) Evolution of total cholesterol concentration measured at day 1, week 8 and week 14 in guinea pigs fed with a standard diet and treated with CRAC 3, 30 mg/kg or its vehicle. C) Evolution of total cholesterol concentration measured at day 1, week 8 and week 14 in guinea pigs fed with a 2% cholesterol diet and treated with CRAC 3, 30 mg/kg or its vehicle. D) Evolution of total cholesterol concentration from day 1 to week 8 and from week 8 to week 14 in percentage of individual variation measured in 2% cholesterol fed guinea pigs. Data shown are means ± SD (n, as indicated in the panels). Results were analyzed by ANOVA followed by Dunnett's test or Student's t test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4. Effect of CRAC treatment on serum free cholesterol levels in guinea pigs fed with a high cholesterol diet. Hartley male guinea pigs were fed with a standard or 2% cholesterol enriched diet for 14 weeks. CRAC treatment started at the end of week 8 until the end of the experiment. Guinea pigs received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 mL/kg) or vehicle every other day. Blood was withdrawn under anesthesia at the study initiation, at the end of weeks 8 and 14 for free cholesterol measurement. Data shown are means ± SD. *** $p < 0.001$ (n, as indicated in the panels). Results were analyzed by ANOVA alone or followed by Student's t test.

Figures 5A-5D. Effect of CRAC treatment on serum LDL and HDL in guinea pigs fed with a high cholesterol diet. Hartley male guinea pigs were fed with standard or 2% cholesterol enriched diet for 14 weeks. CRAC treatment started at the end of week 8 until the end of the experiment. Guinea pig received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 mL/kg) or vehicle every other day. Blood was withdrawn under anesthesia at the study initiation, at the end of week 8 and week 14 for LDL and HDL measurement. A)

Evolution of HDL cholesterol concentration measured at day 1, week 8 and week 14 in guinea pigs fed with a standard or 2% cholesterol diet. B) Evolution of HDL cholesterol concentration measured at week 14 in guinea pigs fed with 2% cholesterol diet and treated with CRAC (3 or 30 mg/kg) or its vehicle. C) Evolution of LDL cholesterol concentration measured at day 1, week 8 and week 14 in guinea pigs fed with a standard or 2% cholesterol diet. D) Evolution of LDL cholesterol concentration measured at week 14 in guinea pigs fed with a 2% cholesterol diet and treated with CRAC (3 or 30 mg/kg) or vehicle. Data shown are means ± SD (n, as indicated in the panels). Results were analyzed by ANOVA alone or followed by Dunnett's test. *** $p<0.001$.

Figure 6. Effect of CRAC treatment on aortic atheroma formation in guinea pigs fed with a high cholesterol diet. Hartley male guinea pigs were fed with a standard or 2% cholesterol enriched diet for 14 weeks. CRAC treatment started at the end of week 8 until the end of the experiment. Guinea pig received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 mL/kg) or vehicle every other day. At the end of week 14, aortas were collected for histology and lipid histochemistry. A, B) Standard diet; C, D) 2% cholesterol diet; E, F) 2% cholesterol diet treated with CRAC 3 mg/kg; 2% cholesterol diet; G, H) 2% cholesterol diet treated with CRAC 30 mg/kg.

Figure 7. Effect of CRAC treatment on oxidative stress in aortas of guinea pigs fed with a high cholesterol diet. Hartley male guinea pigs were fed with a standard or 2% cholesterol enriched diet for 14 weeks. CRAC treatment started at the end of week 8 until the end of the experiment. Guinea pig received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 ml/kg) or vehicle every other day. At the end of week 14, aortas were collected for immunohistochemistry for nitrosylated proteins, a marker of oxidative stress, detected using an anti-nitrotyrosine antibody. A) Standard diet; B) 2% cholesterol diet; C) 2% cholesterol diet treated with CRAC 3 mg/kg; 2% cholesterol diet; D) 2% cholesterol diet treated with CRAC 30 mg/kg. All pictures were taken under identical conditions.

Figures 8A-8B. Effect of CRAC treatment on serum creatine kinase levels in guinea pigs fed with a high cholesterol diet. Hartley male guinea pigs were fed with a standard or 2% cholesterol enriched diet for 14 weeks. CRAC

treatment started at the end of week 8 until the end of the experiment. Guinea pig received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 mL/kg) or vehicle every other day. Serum creatine kinase was measured at weeks 8 and 14 in guinea pigs fed with a standard and 2% cholesterol diet (A) and at week 14 in 2% cholesterol diet fed guinea pigs treated or not with increasing doses of CRAC (B). Data shown are means ± SD (n, as indicated in the panels). Results were analyzed by ANOVA alone or followed by Student's t test. ** $p < 0.01$, *** $p < 0.001$.

Figures 9A-9B. Effect of CRAC treatment on bile cholesterol levels in guinea pigs fed with a high cholesterol diet. Hartley male guinea pigs were fed with standard or 2% cholesterol enriched diet for 14 weeks. CRAC treatment started at the end of week 8 until the end of the experiment. Guinea pig received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 mL/kg) or vehicle every other day. Bile was withdrawn under anesthesia at the end of week 14 for total cholesterol measurement in guinea pigs fed with a standard and 2% cholesterol diet (A) and at week 14 in 2% cholesterol diet fed guinea pigs treated in the presence or absence of increasing doses of CRAC (B). Data shown are means ± SD (n, as indicated in the panels). Results were analyzed by ANOVA alone or followed by Student's t test. *** $p < 0.001$.

Figure 10. Schematic representation of the entero-hepatic circulation and dietary absorption of cholesterol. Cholesterol is eliminated by the liver through the bile under the form of bile acids and free cholesterol. The bile is stored in the gallbladder or evacuated in the duodenum to be eliminated in the feces or to be reabsorbed in the ileum (50-75%) where it re-enters the blood stream under the form of free cholesterol in chylomicron.

Detailed Description of the Invention

Definitions

A "vector" or "construct" (sometimes referred to as gene delivery or gene transfer "vehicle") refers to a macromolecule or complex of molecules comprising a polynucleotide to be delivered to a host cell, either *in vitro* or *in vivo*. The polynucleotide to be delivered may comprise a coding sequence of interest for gene therapy. Vectors include, for example, viral vectors (such as

adenoviruses, adeno-associated viruses (AAV), lentiviruses, herpesvirus and retroviruses), liposomes and other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a polynucleotide to a host cell. Vectors can also comprise other components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. Such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector nucleic acid by the cell; components that influence localization of the polynucleotide within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the polynucleotide. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities. A large variety of such vectors are known in the art and are generally available. When a vector is maintained in a host cell, the vector can either be stably replicated by the cells during mitosis as an autonomous structure, incorporated within the genome of the host cell, or maintained in the host cell's nucleus or cytoplasm.

A "recombinant viral vector" refers to a viral vector comprising one or more heterologous genes or sequences. Since many viral vectors exhibit size constraints associated with packaging, the heterologous genes or sequences are typically introduced by replacing one or more portions of the viral genome. Such viruses may become replication-defective, requiring the deleted function(s) to be provided in *trans* during viral replication and encapsidation (by using, e.g., a helper virus or a packaging cell line carrying genes necessary for replication and/or encapsidation). Modified viral vectors in which a polynucleotide to be delivered is carried on the outside of the viral particle have also been described.

"Gene delivery," "gene transfer," and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred

to as a "transgene") into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of "naked" polynucleotides (such as electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art.

As used herein, the term "cell line" or "host cell" is intended to refer to well-characterized homogenous, biologically pure populations of cells. These cells may be eukaryotic cells that are neoplastic or which have been "immortalized" *in vitro* by methods known in the art, as well as primary cells, or prokaryotic cells. The cell line or host cell may be of mammalian origin or of non-mammalian origin, including plant, insect, yeast, fungal or bacterial sources.

"Transfected" or "transformed" is used herein to include any host cell or cell line, the genome of which has been altered or augmented by the presence of at least one recombinant DNA sequence, which DNA is also referred to in the art of genetic engineering as "heterologous DNA," "exogenous DNA," "genetically engineered," "non-native," or "foreign DNA," wherein said DNA was isolated and introduced into the genome of the host cell or cell line by the process of genetic engineering. The host cells of the present invention are typically produced by transfection with a DNA sequence in a plasmid expression vector, a viral expression vector, or as an isolated linear DNA sequence.

By "transgene" is meant any piece of a nucleic acid molecule (for example, DNA) which is inserted by artifice into a cell either transiently or permanently, and becomes part of the organism if integrated into the genome or maintained extrachromosomally. Such a transgene includes at least a portion of an open reading frame of a gene which is partly or entirely heterologous (i.e.,

foreign) to the transgenic organism, or may represent an open reading frame or a portion thereof of a gene homologous to an endogenous gene of the organism, which portion optionally encodes a polypeptide with substantially the same activity as the corresponding full length polypeptide, e.g., wild-type polypeptide, or at least one activity of the corresponding full length polypeptide.

By "transgenic cell" is meant a cell containing a transgene. For example, a cell transformed with a vector containing an expression cassette can be used to produce a population of cells having altered phenotypic characteristics. A "recombinant cell" is one which has been genetically modified, e.g., by insertion, deletion or replacement of sequences in a nonrecombinant cell by genetic engineering.

The term "wild-type" or "native" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

The term "transduction" denotes the delivery of a polynucleotide to a recipient cell either *in vivo* or *in vitro*, via a viral vector and preferably via a replication-defective viral vector.

The term "heterologous" as it relates to nucleic acid sequences such as gene sequences and control sequences, denotes sequences that are not normally joined together, and/or are not normally associated with a particular cell. Thus, a "heterologous" region of a nucleic acid construct or a vector is a segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid construct could include a coding sequence flanked by sequences not found in association with the coding sequence in nature, i.e., a heterologous promoter. Another example of a heterologous coding sequence is a

construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Similarly, a cell transformed with a construct which is not normally present in the cell would be considered heterologous for purposes of this invention.

By "DNA" is meant a polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in double-stranded or single-stranded form found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having the sequence complementary to the mRNA). The term captures molecules that include the four bases adenine, guanine, thymine, or cytosine, as well as molecules that include base analogues which are known in the art.

As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

DNA molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotide or polynucleotide is referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or

polynucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

A "gene," "polynucleotide," "coding region," "sequence, " "segment," "fragment," or "transgene" which "encodes" a particular protein, is a nucleic acid molecule which is transcribed and optionally also translated into a gene product, e.g., a polypeptide, *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The coding region may be present in either a cDNA, genomic DNA, or RNA form. When present in a DNA form, the nucleic acid molecule may be single-stranded (i.e., the sense strand) or double-stranded. The boundaries of a coding region are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A gene can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the gene sequence.

The term "control elements" refers collectively to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, splice junctions, and the like, which collectively provide for the replication, transcription, post-transcriptional processing and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell.

The term "promoter" is used herein in its ordinary sense to refer to a nucleotide region comprising a DNA regulatory sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA

polymerase and initiating transcription of a downstream (3' direction) coding sequence.

By "enhancer" is meant a nucleic acid sequence that, when positioned proximate to a promoter, confers increased transcription activity relative to the transcription activity resulting from the promoter in the absence of the enhancer domain.

By "operably linked" with reference to nucleic acid molecules is meant that two or more nucleic acid molecules (e.g., a nucleic acid molecule to be transcribed, a promoter, and an enhancer element) are connected in such a way as to permit transcription of the nucleic acid molecule. "Operably linked" with reference to peptide and/or polypeptide molecules is meant that two or more peptide and/or polypeptide molecules are connected in such a way as to yield a single polypeptide chain, i.e., a fusion polypeptide, having at least one property of each peptide and/or polypeptide component of the fusion. The fusion polypeptide is preferably chimeric, i.e., composed of heterologous molecules.

"Homology" refers to the percent of identity between two polynucleotides or two polypeptides. The correspondence between one sequence and to another can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single strand-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two polypeptide, sequences are "substantially homologous" to each other when at least about 80%, preferably at least about 90%, and most preferably at least about 95% of the nucleotides, or amino acids, respectively match over a defined length of the molecules, as determined using the methods above.

By "mammal" is meant any member of the class *Mammalia* including, without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats

and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, rabbits and guinea pigs, and the like.

By "derived from" is meant that a nucleic acid molecule was either made or designed from a parent nucleic acid molecule, the derivative retaining substantially the same functional features of the parent nucleic acid molecule, e.g., encoding a gene product with substantially the same activity as the gene product encoded by the parent nucleic acid molecule from which it was made or designed.

By "expression construct" or "expression cassette" is meant a nucleic acid molecule that is capable of directing transcription. An expression construct includes, at the least, a promoter. Additional elements, such as an enhancer, and/or a transcription termination signal, may also be included.

The term "exogenous," when used in relation to a protein, gene, nucleic acid, or polynucleotide in a cell or organism refers to a protein, gene, nucleic acid, or polynucleotide which has been introduced into the cell or organism by artificial or natural means, or in relation a cell refers to a cell which was isolated and subsequently introduced to other cells or to an organism by artificial or natural means. An exogenous nucleic acid may be from a different organism or cell, or it may be one or more additional copies of a nucleic acid which occurs naturally within the organism or cell. An exogenous cell may be from a different organism, or it may be from the same organism. By way of a non-limiting example, an exogenous nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature.

The term "isolated" when used in relation to a nucleic acid, peptide, polypeptide or virus refers to a nucleic acid sequence, peptide, polypeptide or virus that is identified and separated from at least one contaminant nucleic acid, polypeptide or other biological component with which it is ordinarily associated in its natural source. Isolated nucleic acid, peptide, polypeptide or virus is present in a form or setting that is different from that in which it is found in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a

mixture with numerous other mRNAs that encode a multitude of proteins. The isolated nucleic acid molecule may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the molecule will contain at a minimum the sense or coding strand (i.e., the molecule may single-stranded), but may contain both the sense and anti-sense strands (i.e., the molecule may be double-stranded).

The term "recombinant DNA molecule" as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule that is expressed from a recombinant DNA molecule.

The term "peptide", "polypeptide" and protein" are used interchangeably herein unless otherwise distinguished.

The term "sequence homology" means the proportion of base matches between two nucleic acid sequences or the proportion amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length of a selected sequence that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used, 6 bases or less are preferred with 2 bases or less more preferred. When using oligonucleotides as probes or treatments, the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and more preferably not less than 19 matches out of 20 possible base pair matches (95%).

The term "selectively hybridize" means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments of the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein.

Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest is at least 65%, and more typically with preferably increasing homologies of at least about 70%, about 90%, about 95%, about 98%, and 100%.

Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff, 1972. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program.

The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene

sequence given in a sequence listing, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity.

A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981), by the homology alignment algorithm of Needleman and Wunsch (1970), by the search for similarity method of Pearson and Lipman (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total

number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denote a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 20-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least about 80 percent sequence identity, preferably at least about 90 percent sequence identity, more preferably at least about 95 percent sequence identity, and most preferably at least about 99 percent sequence identity. In one embodiment, a peptide useful in the methods of the invention has at least 80%, 85%, 90%, 95% or more, amino acid sequence identity with one of SEQ ID NOs.1-24

As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, about 90%, about 95%, and about 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

The term "therapeutically effective amount" is meant to refer to the amount necessary to reduce symptoms or cure the individual.

I. Preparation of Peptides and Polypeptides and Nucleic Acid Molecules of the Invention

A. Nucleic Acid Molecules

1. Expression Cassettes

To prepare expression cassettes for transformation, the recombinant DNA sequence or segment encoding a peptide or polypeptide having a cholesterol recognition/interaction amino acid sequence may be circular or linear, double-stranded or single-stranded. A DNA sequence which encodes an RNA sequence that is substantially complementary to a mRNA sequence is typically a "sense" DNA sequence cloned into a cassette in the opposite orientation (i.e., 3′ to 5′ rather than 5′ to 3′). Generally, the recombinant DNA sequence or segment is in the form of chimeric DNA, such as plasmid DNA, that can also contain coding regions flanked by control sequences which promote the expression of the DNA present in the resultant host cell.

As used herein, "chimeric" nucleic acid means that a vector comprises DNA from at least two different species, is synthetic or comprises DNA from the same species which is linked or associated in a manner which does not occur in the "native" or wild type of the species.

Aside from DNA sequences that serve as transcription units, a portion of the recombinant DNA may be untranscribed, serving a regulatory or a structural function. For example, the DNA may itself comprise a promoter that is active in prokaryotic or eukaryotic cells such as mammalian cells, or may utilize a promoter already present in the genome that is the transformation target.

Other elements functional in the host cells, such as introns, enhancers, polyadenylation sequences and the like, may also be a part of the recombinant DNA. Such elements may or may not be necessary for the function of the DNA, but may provide improved expression of the DNA by affecting transcription, stability of the mRNA, or the like. Such elements may be included in the DNA as desired to obtain the optimal performance of the transforming DNA in the cell.

The recombinant DNA to be introduced into the cells may further contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of transformed cells from the population of cells sought to be transformed. Alternatively, the selectable marker may be carried on a separate piece of DNA and used in a co-transformation procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers are well known in the art and include, for example, antibiotic and herbicide-resistance genes, such as *neo, hpt, dhfr, bar, aroA, dapA* and the like. See also, the genes listed on Table 1 of Lundquist et al. (U.S. Patent No. 5,848,956).

Reporter genes are used for identifying potentially transformed cells and for evaluating the functionality of regulatory sequences. Reporter genes which encode for easily assayable proteins are well known in the art. In general, a reporter gene is a gene which is not present in or expressed by the recipient organism or tissue and which encodes a protein whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Preferred genes include the chloramphenicol acetyl transferase gene (cat) from Tn9 of *E. coli*, the beta-glucuronidase gene (gus) of the *uidA* locus of *E. coli*, and the luciferase gene from firefly *Photinus pyralis*. Expression of the reporter gene is assayed at a suitable time after the recombinant DNA has been introduced into the recipient cells.

The general methods for constructing recombinant DNA which can transform target cells are well known to those skilled in the art, and the same compositions and methods of construction may be utilized to produce the DNA useful herein.

2. Transformation into Host Cells

The recombinant DNA can be readily introduced into the host cells, e.g., mammalian, bacterial, yeast or insect cells by transfection with an expression vector comprising DNA or its complement, by any procedure useful for the introduction into a particular cell, e.g., physical or biological methods, to yield a transformed cell having the recombinant DNA stably integrated into its genome,

so that the DNA molecules, sequences, or segments, of the present invention are expressed by the host cell.

Physical methods to introduce a recombinant DNA into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Biological methods to introduce the DNA of interest into a host cell include the use of DNA and RNA viral vectors. The main advantage of physical methods is that they are not associated with pathological or oncogenic processes of viruses. However, they are less precise, often resulting in multiple copy insertions, random integration, disruption of foreign and endogenous gene sequences, and unpredictable expression. For mammalian cells, it is desirable to use an efficient means of precisely inserting a single copy gene into the host genome. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like.

To confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular endonuclease, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify molecules falling within the scope of the invention.

To detect and quantitate RNA produced from introduced recombinant DNA segments, RT-PCR may be employed. In this application of PCR, it is first necessary to reverse transcribe RNA into DNA, using enzymes such as reverse transcriptase, and then through the use of conventional PCR techniques amplify the DNA. In most instances PCR techniques, while useful, will not demonstrate integrity of the RNA product. Further information about the nature of the RNA product may be obtained by Northern blotting. This technique demonstrates the presence of an RNA species and gives information about the integrity of that RNA. The presence or absence of an RNA species can also be determined using dot or slot blot Northern hybridizations. These techniques are

modifications of Northern blotting and only demonstrate the presence or absence of an RNA species.

While Southern blotting and PCR may be used to detect the recombinant DNA segment in question, they do not provide information as to whether the recombinant DNA segment is being expressed. Expression may be evaluated by specifically identifying the peptide products of the introduced preselected DNA sequences or evaluating the phenotypic changes brought about by the expression of the introduced recombinant DNA segment in the host cell.

B. Peptides and Polypeptides

The isolated peptides or polypeptides having a cholesterol recognition/interaction amino acid sequence can be synthesized *in vitro*, e.g., by the solid phase peptide synthetic method or by recombinant DNA approaches (see above). The solid phase peptide synthetic method is an established and widely used method, which is described in the following references: Stewart et al. (1969); Merrifield (1963); Meienhofer (1973); Bavaay and Merrifield, (1980); and Clark-Lewis et al. (1997). These peptides can be further purified by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on an anion-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; or ligand affinity chromatography.

Once isolated and characterized, derivatives, e.g., chemically derived derivatives, can be readily prepared. For example, amides of the peptide or polypeptide may also be prepared by techniques well known in the art for converting a carboxylic acid group or precursor, to an amide. A preferred method for amide formation at the C-terminal carboxyl group is to cleave the peptide from a solid support with an appropriate amine, or to cleave in the presence of an alcohol, yielding an ester, followed by aminolysis with the desired amine.

Salts of carboxyl groups of a peptide or polypeptide may be prepared in the usual manner by contacting the peptide or polypeptide with one or more equivalents of a desired base such as, for example, a metallic hydroxide base, e.g., sodium hydroxide; a metal carbonate or bicarbonate base such as, for

example, sodium carbonate or sodium bicarbonate; or an amine base such as, for example, triethylamine, triethanolamine, and the like.

N-acyl derivatives of an amino group of the peptide or polypeptide may be prepared by utilizing an N-acyl protected amino acid for the final condensation, or by acylating a protected or unprotected peptide or polypeptide. O-acyl derivatives may be prepared, for example, by acylation of a free hydroxy peptide or peptide resin. Either acylation may be carried out using standard acylating reagents such as acyl halides, anhydrides, acyl imidazoles, and the like. Both N- and O-acylation may be carried out together, if desired.

Formyl-methionine, pyroglutamine and trimethyl-alanine may be substituted at the N-terminal residue of the peptide or polypeptide. Other amino-terminal modifications include aminooxypentane modifications (see Simmons et al., 1997).

In addition, the amino acid sequence of a particular peptide or polypeptide can be modified so as to result in a peptide or polypeptide variant of that particular peptide or polypeptide, e.g., the amino acid sequence of the variant has substantial identity to the reference peptide or polypeptide. The modification includes the substitution of at least one amino acid residue in the peptide for another amino acid residue, including substitutions which utilize the D rather than L form, as well as other well known amino acid analogs, e.g., unnatural amino acids such as α, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and the like. Amino acid analogs include phosphoserine, phosphothreonine, phosphotyrosine, hydroxyproline, gamma-carboxyglutamate; hippuric acid, octahydroindole-2-carboxylic acid, statine, 1,2,3,4,-tetrahydroisoquinoline-3-carboxylic acid, penicillamine, ornithine, citruline, α-methyl-alanine, para-benzoyl-phenylalanine, phenylglycine, propargylglycine, sarcosine, ϵ-N,N,N-trimethyllysine, ϵ-N-acetyllysine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, ω-N-methylarginine, and other similar amino acids and imino acids and tert-butylglycine. Conservative amino acid substitutions are preferred--that is, for example, aspartic-glutamic as acidic amino acids; lysine/arginine/histidine as basic amino acids; leucine/isoleucine/methionine/alanine/valine/glycine as hydrophobic amino acids; serine/threonine as hydrophilic amino acids. Conservative amino acid

substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. For example, it is reasonable to expect that replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting variant polypeptide. Whether an amino acid change results in a functional peptide or polypeptide can readily be determined by assaying the activity of the peptide or polypeptide variant.

Amino acid substitutions falling within the scope of the invention, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide or polypeptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic; trp, tyr, phe.

The invention also envisions peptide or polypeptide variants with non-conservative substitutions. Non-conservative substitutions entail exchanging a member of one of the classes described above for another.

Acid addition salts of amino residues of the peptide or polypeptide may be prepared by contacting the peptide or amine with one or more equivalents of the desired inorganic or organic acid, such as, for example, hydrochloric acid.

Esters of carboxyl groups of the peptides or polypeptides may also be prepared by any of the usual methods known in the art.

Peptide or polypeptide analogs have properties analogous to those of the corresponding peptide. These analogs can be referred to as "peptide mimetics" or "peptidomimetics" and can be developed with the aid of computerized molecular modeling. These analogs include structures having one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, $-CH=CH-$(cis and trans), $-CH=CF-$(trans), $-CoCH_2-$, $-CH(OH)CH_2-$, and $-CH_2SO-$, by methods known in the art and further described in the following references: Spatola (1983); Spatola (1983); Morley (1980); Hudson (1979) ($-CH_2NH-$, CH_2CH_2-); Spatola (1986) ($-CH_2-S$); Hann (1982) ($-CH-CH-$, cis and trans); Almquist (1980) ($-COCH_2-$); Jennings-White et al. (1982) ($-COCH_2-$); EP 45665 ($-CH(OH)CH_2-$); Holladay et al. (1983) ($-C(OH)CH_2-$); and Hruby (1982) ($-CH_2S-$). In one embodiment the non-peptide linkage is $-CH_2NH-$. Such analogs may have greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and be economically prepared. Labeling of analogs usually involves covalent attachment of one or more labels, directly or through a spacer (e.g., an amide group), to non-interfering positions(s) on the analog that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the macromolecule(s) to which the analog specifically binds to produce the desired effect. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may also be used to generate more stable peptides.

II. Exemplary Cholesterol Recognition/Interaction Sequences

The present invention provides a consensus sequence for the recognition/interaction of/with cholesterol having formula (I)

$$Z\text{-}(X)_{0\text{-}5}\text{-}B\text{-}(X)_{0\text{-}5}\text{-}J$$

wherein Z represents a neutral and hydrophobic amino acid, such as Leucine or Valine, B represents a neutral and polar amino acid, such as Tyrosine, J

represents a basic amino acid, such as Arginine or Lysine and X represents any amino acid or an isolated peptide having a cholesterol recognition/interaction amino acid sequence having formula (II) U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

In one embodiment, the present invention relates to a minimum amino acid sequence specific for recognition/interaction with cholesterol, namely the amino acid sequence

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z represents a neutral and hydrophobic amino acid, such as Leucine, Valine, Alanine, Isoleucine, Methionine, Phenylalanine and Tryptophan, B represents a neutral and polar amino acid, such as Tyrosine, Threonine, Serine, Glycine, Glutamine, Cysteine, Asparagine, J represents a basic amino acid, such as Arginine, Lysine, or arginine, and X represents any amino acids selected from the group consisting of Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic acid (Asp, D), Cystein (Cys, C), Glutamine (Gln, Q), Glutamic acid (Glu, E), Glycine (Gly, G) , Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V). For example, some of the sequences useful in the methods of the invention include but are not limited to Leu-Asn-Tyr-Tyr-Val-Trp-Arg (SEQ ID NO:2), Leu-Asn-Tyr-Cys-Val-Trp-Arg (SEQ ID NO:3), Leu-Asn-Tyr-Arg (SEQ ID NO:4), or Val-Ala-Tyr-His-Gln-Tyr-Tyr-Gln-Arg (SEQ ID NO:5).

In one embodiment, the number of X amino acids can be from none (zero) to five, e.g., 1 to 5, 2 to 5, or 3 to 5 amino acids, or can be from zero to ten, e.g., 1 to 6, 2 to 7, or 3 to 9 amino acids.

The cholesterol recognition/interaction amino acid sequence consensus pattern was found in molecules shown or suggested to interact with cholesterol, such as apolipoprotein A-1 (Boyle et al., 1992), caveolin (Murata et al., 1995), DBI (Papadopoulos, 1993; Papadopoulos, 1998), steroidogenesis acute regulatory protein (StAR) (Stocco and Clark, 1996), hedgehog protein (Porter et al. 1996), cytochrome P450 C26/25 (Su et al., 1990), annexin II (Harder et al., 1997), sterol carrier protein-2 (Colles et al. 1995), cholesterol 7α-monooxygenase (Kai et al. 1995), cholesterol oxidase (Ishizaki et al. 1991),

cholesterol dehydrogenase (Horinouchi et al. 1991), bile-salt-activated lipase precursor (cholesterol esterase) (Nilsson et al. 1990), and acyl-CoA cholesterol acyltransferase (Pape et al. 1995) as shown in Table 1.

Table 1

Mouse PBR	VLNYYVWR (SEQ ID NO:6)
Rat PBR	LNYYVWR (SEQ ID NO:7)
Human PBR	LNYCVWR (SEQ ID NO:8)
Bovine PBR	LNYR (SEQ ID NO:9)
Rat P450scc	VAYHQYYQR (SEQ ID NO:10)
Human P450scc	VAYHQYYQR (SEQ ID NO:11)
Pig P450scc	VAYHZHYQK (SEQ ID NO:12)
Mouse apolipoprotein A-I	LNEYHTR (SEQ ID NO:13)
Mouse caveolin	VTKYWFYR (SEQ ID NO:14)
Human hedgehog	VFYVIETR (SEQ ID NO:15)
Mouse DBI	LFIYSHFK (SEQ ID NO:16)
Mouse StAR	LCAGSSYRHMR (SEQ ID NO:17)
Rat Annexin II	VYKEMYKTDLEK (SEQ ID NO:18)
Rat P450c26/25	VLCTYVVSR (SEQ ID NO:19)
Strept. cholesterol oxidase	VSLYLAITK (SEQ ID NO:20)
Mouse cholesterol 7"-monooxygenase	VTEGMYAFCYR (SEQ ID NO:21)
Nocardia cholesterol dehydrogenase	VTEAYRQR (SEQ ID NO:22)
Human Bile-Salt-Activated-Lipase	LSPYNKGLIR (SEQ ID NO:23)
Rabbit acyl-CoA cholesterol acyltransferase	VVDYIDEGR (SEQ ID NO:24)

III. Formulations and Routes of Administration of Agents Having a Cholesterol Recognition/Interaction Amino Acid Sequence

Agents having a cholesterol recognition/interaction amino acid sequence are preferably administered, at dosages of at least about 0.01 to about 0.1, more preferably about 0.02 to about 0.075, and even more preferably about 0.02 to about 0.03 mmol/kg, although other dosages may provide beneficial results. The amount administered will vary depending on various factors including, but not

limited to, the agent chosen, the target organ or tissue and if the agent is modified for cell, organ or tissue targeting, bioavailability and/or *in vivo* stability.

Administration of the agents in accordance with the present invention may be continuous or intermittent, depending, for example, upon the recipient's physiological condition, and other factors known to skilled practitioners. The administration of the agents of the invention may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated.

One or more suitable unit dosage forms comprising the agents of the invention, which, as discussed below, may optionally be formulated for sustained release, can be administered by a variety of routes including oral, or parenteral, including by rectal, buccal, vaginal and sublingual, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, intrathoracic, intrapulmonary and intranasal routes. The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to pharmacy. Such methods may include the step of bringing into association the agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

When the agents are prepared for oral administration, they are preferably combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. The total active ingredients in such formulations comprise from 0.1 to 99.9% by weight of the formulation. By "pharmaceutically acceptable" it is meant the carrier, diluent, excipient, and/or salt must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof. The active ingredient for oral administration may be present as a powder or as granules; as a solution, a suspension or an emulsion; or in achievable base such as a synthetic resin for ingestion of the active ingredients from a chewing gum. The active ingredient may also be presented as a bolus, electuary or paste.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, douches, lubricants, foams or sprays containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate. Formulations suitable for rectal administration may be presented as suppositories.

Pharmaceutical formulations containing the agents can be prepared by procedures known in the art using well known and readily available ingredients. For example, the agent can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose, HPMC and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

For example, tablets or caplets containing the agents can include buffering agents such as calcium carbonate, magnesium oxide and magnesium carbonate. Caplets and tablets can also include inactive ingredients such as cellulose, pregelatinized starch, silicon dioxide, hydroxy propyl methyl cellulose, magnesium stearate, microcrystalline cellulose, starch, talc, titanium dioxide, benzoic acid, citric acid, corn starch, mineral oil, polypropylene glycol, sodium phosphate, and zinc stearate, and the like. Hard or soft gelatin capsules containing an agent of the invention can contain inactive ingredients such as gelatin, microcrystalline cellulose, sodium lauryl sulfate, starch, talc, and titanium dioxide, and the like, as well as liquid vehicles such as polyethylene glycols (PEGs) and vegetable oil. Moreover, enteric coated caplets or tablets of an agent of the invention are designed

to resist disintegration in the stomach and dissolve in the more neutral to alkaline environment of the duodenum.

The agents can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes.

The pharmaceutical formulations of the agents can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an emulsion or suspension.

Thus, the agent may be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The active ingredients may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

These formulations can contain pharmaceutically acceptable vehicles and adjuvants which are well known in the prior art. It is possible, for example, to prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint, chosen, in addition to water, from solvents such as acetone, ethanol, isopropyl alcohol, glycol ethers such as the products sold under the name "Dowanol", polyglycols and polyethylene glycols, C_1-C_4 alkyl esters of short-chain acids, preferably ethyl or isopropyl lactate, fatty acid triglycerides such as the products marketed under the name "Miglyol", isopropyl myristate, animal, mineral and vegetable oils and polysiloxanes.

The compositions according to the invention can also contain thickening agents such as cellulose and/or cellulose derivatives. They can also contain gums such as xanthan, guar or carbo gum or gum arabic, or alternatively polyethylene glycols, bentones and montmorillonites, and the like.

It is possible to add, if necessary, an adjuvant chosen from antioxidants, surfactants, other preservatives, film-forming, keratolytic or comedolytic agents, perfumes and colorings. Also, other active ingredients may be added, whether for the conditions described or some other condition.

For example, among antioxidants, t-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene and α-tocopherol and its derivatives may be mentioned. The galenical forms chiefly conditioned for topical application take the form of creams, milks, gels, dispersion or microemulsions, lotions thickened to a greater or lesser extent, impregnated pads, ointments or sticks, or alternatively the form of aerosol formulations in spray or foam form or alternatively in the form of a cake of soap.

Additionally, the agents are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal or respiratory tract, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances, such as polylactide-glycolates, liposomes, microemulsions, microparticles, nanoparticles, or waxes. These coatings, envelopes, and protective matrices are useful to coat indwelling devices, e.g., stents, catheters, peritoneal dialysis tubing, and the like.

The agents of the invention can be delivered via patches for transdermal administration. See U.S. Patent No. 5,560,922 for examples of patches suitable for transdermal delivery of an agent. Patches for transdermal delivery can comprise a backing layer and a polymer matrix which has dispersed or dissolved therein an agent, along with one or more skin permeation enhancers. The backing layer can be made of any suitable material which is impermeable to the agent. The backing layer serves as a protective cover for the matrix layer and provides also a support function. The backing can be formed so that it is essentially the same size layer as the polymer matrix or it can be of larger dimension so that it can extend beyond the side of the polymer matrix or overlay the side or sides of the polymer matrix and then can extend outwardly in a manner that the surface of the extension of the backing layer can be the base for an adhesive means. Alternatively, the polymer

matrix can contain, or be formulated of, an adhesive polymer, such as polyacrylate or acrylate/vinyl acetate copolymer. For long-term applications it might be desirable to use microporous and/or breathable backing laminates, so hydration or maceration of the skin can be minimized.

Examples of materials suitable for making the backing layer are films of high and low density polyethylene, polypropylene, polyurethane, polyvinylchloride, polyesters such as poly(ethylene phthalate), metal foils, metal foil laminates of such suitable polymer films, and the like. Preferably, the materials used for the backing layer are laminates of such polymer films with a metal foil such as aluminum foil. In such laminates, a polymer film of the laminate will usually be in contact with the adhesive polymer matrix.

The backing layer can be any appropriate thickness which will provide the desired protective and support functions. A suitable thickness will be from about 10 to about 200 microns.

Generally, those polymers used to form the biologically acceptable adhesive polymer layer are those capable of forming shaped bodies, thin walls or coatings through which agents can pass at a controlled rate. Suitable polymers are biologically and pharmaceutically compatible, nonallergenic and insoluble in and compatible with body fluids or tissues with which the device is contacted. The use of soluble polymers is to be avoided since dissolution or erosion of the matrix by skin moisture would affect the release rate of the agents as well as the capability of the dosage unit to remain in place for convenience of removal.

Exemplary materials for fabricating the adhesive polymer layer include polyethylene, polypropylene, polyurethane, ethylene/propylene copolymers, ethylene/ethylacrylate copolymers, ethylene/vinyl acetate copolymers, silicone elastomers, especially the medical-grade polydimethylsiloxanes, neoprene rubber, polyisobutylene, polyacrylates, chlorinated polyethylene, polyvinyl chloride, vinyl chloride-vinyl acetate copolymer, crosslinked polymethacrylate polymers (hydrogen), polyvinylidene chloride, poly(ethylene terephthalate), butyl rubber, epichlorohydrin rubbers, ethylene-vinyl alcohol copolymers, ethylene-vinyloxyethanol copolymers; silicone copolymers, for example, polysiloxane-

polycarbonate copolymers, polysiloxane-polyethylene oxide copolymers, polysiloxane-polymethacrylate copolymers, polysiloxane-alkylene copolymers (e.g., polysiloxane-ethylene copolymers), polysiloxane-alkylenesilane copolymers (e.g., polysiloxane-ethylenesilane copolymers), and the like; cellulose polymers, for example methyl or ethyl cellulose, hydroxy propyl methyl cellulose, and cellulose esters; polycarbonates; polytetrafluoroethylene; and the like.

Preferably, a biologically acceptable adhesive polymer matrix should be selected from polymers with glass transition temperatures below room temperature. The polymer may, but need not necessarily, have a degree of crystallinity at room temperature. Cross-linking monomeric units or sites can be incorporated into such polymers. For example, cross-linking monomers can be incorporated into polyacrylate polymers, which provide sites for cross-linking the matrix after dispersing the agent into the polymer. Known cross-linking monomers for polyacrylate polymers include polymethacrylic esters of polyols such as butylene diacrylate and dimethacrylate, trimethylol propane trimethacrylate and the like. Other monomers which provide such sites include allyl acrylate, allyl methacrylate, diallyl maleate and the like.

Preferably, a plasticizer and/or humectant is dispersed within the adhesive polymer matrix. Water-soluble polyols are generally suitable for this purpose. Incorporation of a humectant in the formulation allows the dosage unit to absorb moisture on the surface of skin which in turn helps to reduce skin irritation and to prevent the adhesive polymer layer of the delivery system from failing.

Agents released from a transdermal delivery system must be capable of penetrating each layer of skin. In order to increase the rate of permeation of an agent, a transdermal drug delivery system must be able in particular to increase the permeability of the outermost layer of skin, the stratum corneum, which provides the most resistance to the penetration of molecules. The fabrication of patches for transdermal delivery of agents is well known to the art.

For administration to the upper (nasal) or lower respiratory tract by inhalation, the agents of the invention are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering

an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of the agent and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatine or blister packs from which the powder may be administered with the aid of an inhalator, insufflator or a metered-dose inhaler.

For intra-nasal administration, the agent may be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker).

The local delivery of the agents of the invention can also be by a variety of techniques which administer the agent at or near the site of disease. Examples of site-specific or targeted local delivery techniques are not intended to be limiting but to be illustrative of the techniques available. Examples include local delivery catheters, such as an infusion or indwelling catheter, e.g., a needle infusion catheter, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct applications.

For topical administration, the agents may be formulated as is known in the art for direct application to a target area. Conventional forms for this purpose include wound dressings, coated bandages or other polymer coverings, ointments, creams, lotions, pastes, jellies, sprays, and aerosols, as well as in toothpaste and mouthwash, or by other suitable forms, e.g., via a coated condom. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The active ingredients can also be delivered via iontophoresis, e.g., as disclosed in U.S. Patent Nos. 4,140,122; 4,383,529; or

4,051,842. The percent by weight of an agent of the invention present in a topical formulation will depend on various factors, but generally will be from 0.01% to 95% of the total weight of the formulation, and typically 0.1-25% by weight.

When desired, the above-described formulations can be adapted to give sustained release of the active ingredient employed, e.g., by combination with certain hydrophilic polymer matrices, e.g., comprising natural gels, synthetic polymer gels or mixtures thereof.

Drops, such as eye drops or nose drops, may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs. Drops can be delivered via a simple eye dropper-capped bottle, or via a plastic bottle adapted to deliver liquid contents dropwise, via a specially shaped closure.

The agent may further be formulated for topical administration in the mouth or throat. For example, the active ingredients may be formulated as a lozenge further comprising a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia; mouthwashes comprising the composition of the present invention in a suitable liquid carrier; and pastes and gels, e.g., toothpastes or gels, comprising the composition of the invention.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In

addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration.

The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions are prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the active ingredient. The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefor.

Veterinary carriers are materials useful for the purpose of administering the composition and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient.

These veterinary compositions may be administered orally, parenterally or by any other desired route.

Effective dose of active ingredient depends at least on the nature of the condition being treated, toxicity, whether the compound is being used prophylactically (lower doses), the method of delivery, and the pharmaceutical formulation, and will be determined by the clinician using conventional dose escalation studies. It can be expected to be from about 0.0001 to about 100 mg/kg body weight per day. Typically, from about 0.01 to about 10 mg/kg body weight per day. More typically, from about .01 to about 5 mg/kg body weight per day. More typically, from about .05 to about 0.5 mg/kg body weight per day. For example, the daily candidate dose for an adult human of approximately 70 kg body weight will range from 1 mg to 1000 mg, preferably between 5 mg and 500 mg, and may take the form of single or multiple doses. The compound may be conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form. Useful dosages of the compounds of the invention can be determined by comparing their *in vitro* activity and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

The invention will be described by the following nonlimiting example.

Example

The peripheral-type benzodiazepine receptor (PBR) is a high affinity cholesterol and drug binding protein located in the outer mitochondrial membrane of steroidogenic cells where it mediates the transfer of cholesterol into the mitochondria, the rate-determining step in steroidogenesis. A domain in the carboxy-terminus of PBR was identified and characterized as the cholesterol recognition/interaction amino acid consensus (CRAC). The ability of the CRAC domain to bind to cholesterol led to the hypothesis that this peptide could be used as a potential hypocholesterolemic agent. As described below, the administration of the VLNYYVWR (SEQ ID NO:1) CRAC sequence to two different

hypercholesterolemic animal models, the ApoE knock-out B6.129P2-Apoetm1Unc/J mice and the 2% cholesterol diet fed guinea pigs, resulted in reduced circulating cholesterol levels. Detailed studies in the high cholesterol diet fed guinea pig model indicated that CRAC treatment induced an increase in circulating HDL, reduced cholesterol levels in bile and completely prevented the formation of atheroma deposits in the aorta. In parallel, CRAC treatment also prevented the high cholesterol diet–induced increase in serum creatine kinase, suggesting that it protected the animals against the high cholesterol-induced cardiac and muscular suffering. Taken together these results indicate that CRAC may constitute a treatment of hypercholesterolemia and atherosclerosis.

Materials and Methods

Cholesterol-Protein Binding Blot Assay

The interaction between apolipoprotein bound cholesterol and CRAC was studied using the cholesterol-protein binding blot assay (CPBBA) as described in Yao et al., 2003. In brief, 2 μg of isolated lipoproteins (ApoE and ApoAI) were incubated with ^{3}H-cholesterol (0.1 μCi for ApoE and 0.02 μCi for ApoAI) in the presence or absence of increasing concentrations of the carboxy-terminal domain of PBR (PBR-CTF, e.g., which domain may include residues 144 to 169 of PBR, or a portion thereof) containing the CRAC peptide in 20 μL volume at 37°C for 1 hour for ApoE or 3 hours for ApoAI. Optimal incubation times and cholesterol concentrations were determined in preliminary experiments. At the end of the incubation time, the samples were separated by 1.5% agarose (Type I-B, Sigma) gel electrophoresis and transferred to nitrocellulose membrane in 10XSSC buffer. The membrane was exposed to a tritium-sensitive screen and analyzed by phosphoimaging using the Cyclone Storage phosphor system (Packard BioScience). Image-densitometric analysis of the radioactivity was performed as described above. This method allowed for the separation, visualization and identification of CRAC and apoE which have incorporated radiolabeled cholesterol under native conditions. Low molecular weight unincorporated cholesterol is separated and eliminated during electrophoresis.

In Vivo Studies

All experimental protocols involving animals were approved by the Georgetown University Animal Care and Use Committee. Experiments were performed according to the code of practice for animal experimentation of the Animal Welfare Act and the Public Health Service Policy on Laboratory Animal Care.

ApoE knock-out B6.129P2-Apoetm1Unc/J mice (Jackson Laboratory, Bar Harbor, MN) weighing 18-22 grams at the study initiation received one daily intraperitoneal (i.p.) injection of CRAC solution at 10, 30 and 100 mg/kg (0.15 mL/kg) or vehicle for 14 days. At the end of the treatment period, blood was withdrawn by cardiac puncture for cholesterol measurement.

Hartley male guinea pigs weighing 400 grams at the study initiation were fed with a standard or 2% cholesterol enriched diet for 14 weeks. CRAC treatment started at the end of week 8 until the end of the experiment. Guinea pig received one i.p. injection of CRAC solution at 3 and 30 mg/kg (2.5 mL/kg) or vehicle every other day. Blood was withdrawn under anesthesia (isoflurane 3%) at the study initiation, at the end of week 8 and week 14 to determine total cholesterol, free cholesterol, LDL and HDL levels. Creatine kinase was measured at weeks 8 and 14. At the end of week 14, hearts and aorta were collected for histology purposes as well as the gallbladder content for cholesterol measurement. Aortas were collected at week 14 for histology purposes.

Cholesterol, HDL and LDL Measurements

Total and free cholesterol was measured in serum and bile salts using Amplex® Red Cholesterol Assay Kit (Molecular Probes, Eugene, OR) according to the manufacturer's recommendations. Guinea pig HDL-cholesterol, LDL-cholesterol and creatine kinase (CK) were measured by Antech Inc. (Morrisville, NC) using proprietary methods. The HDL-cholesterol test used was a two reagent homogenous system for the selective measurement of serum HDL-cholesterol in the presence of other lipoprotein particles that include total cholesterol. The LDL-cholesterol test used was based on an enzymatic selective protection methodology. CK levels were determined using an enzymatic reaction based on the ability of CK

to reversibly catalyze the transfer of a phosphate group from creatine phosphate to adenosine diphosphate to give creatine and adenosine triphosphate as products.

Histochemistry & Immunohistochemistry

Guinea pig aortas were collected, embedded and cut at 20 μm thickness. Lipid deposits were detected by oil red staining and general morphology visualized by Mayer's hematoxylin staining. Nitrosylated proteins were detected using an anti-nitrotyrosine antibody (1/50) (Chemicon, Temecula, CA) and anti-mouse Alexafluor 488 secondary antibody (1/200) (Molecular Probes).

Statistical Analysis

Data were analyzed by ANOVA followed by Dunnett's test or ANOVA followed by Student's t test. Results are presented as means ± SD.

Results

Cholesterol Displacement from ApoE and ApoA1

The PBR-CTF containing the CRAC domain affected the binding of cholesterol to ApoE (Figure 1A) and ApoA1 (Figure 1C) suggesting that CRAC could remove cholesterol from the lipoproteins VLDL and HDL or alter the density of the formed complexes, as shown by the change in the migratory pattern of the radiolabeled cholesterol-protein complex in the presence and absence of increasing concentrations of PBR-CTF. As expected from previous studies (Li and Papadopoulos, 1998; Li et al., 2001), PBR-CTF having amino acids responsible for the interaction with cholesterol replaced, failed to affect the cholesterol-ApoE and cholesterol-ApoAI interactions (Figures 1B and 1D), thus confirming the importance of the CRAC sequence to affect cholesterol-lipoprotein interaction.

Serum Cholesterol Level Measurement in ApoE Knock-Out B6.129P2-Apoetm1Unc/J Mice

CRAC peptide treatment decreased in a dose-dependent manner circulating cholesterol levels in ApoE knock-out B6.129P2-Apoetm1Unc/J mice (Figure 2). The doses of 10, 30 and 100 mg/kg decreased the cholesterol serum level by 10% (n = 12), 17% (n = 11) and 21% (n = 11), respectively, compared to the control (n = 11) (F ratio 5.896, Prob > F 0.001).

Serum Cholesterol Level Measurement in Hartley Male Guinea Pigs

A diet containing 2% cholesterol induced an increase of serum cholesterol levels in guinea pigs, measured at week 8, compared to the guinea pigs fed with a standard diet (87.31 ± 8.37, n = 6, versus 57.17 ± 3.90 mg/dl, n = 3, $p < 0.001$). This difference was more pronounced after 14 weeks (215.46 ± 61.13, n = 7, versus 25.33 ± 4.74 mg/dl, n = 3, $p < 0.001$) (Figure 3A). CRAC treatment had no effect on basal cholesterol level as measured in standard diet fed guinea pig serum (Figure 3B). The observed increase between day 1 and week 8 was comparable between the three groups fed with the 2% cholesterol diet (60-80%, Figure 3D). CRAC treatment reduced in a dose-dependent manner the diet-induced total cholesterol increase measured at week 14 (Figure 3C). Compared with the average of individual variation of total cholesterol levels between weeks 8 and 14, the increase observed in the control group was 168.51% (n = 6) (Figure 3D). This cholesterol level increase was significantly lowered by CRAC 3 mg/kg (105.97 ± 37.24%, n = 8, $p < 0.05$) and CRAC 30 mg/kg (34.01 ± 32.56%, n = 7, $p < 0.001$).

The effect of CRAC treatment on free cholesterol paralleled the effect of the treatment on total cholesterol. The 2% cholesterol diet induced a dramatic increase of free cholesterol in guinea pig serum compared to the standard diet (87.96 ± 24.93 mg/dl, n = 6, versus 15.81 ± 1.07 mg/dl, n = 3, $p < 0.001$) (Figure 4). CRAC treatment reduced in a dose-dependent manner the diet-induced cholesterol increase (F Ratio = 9.819, Prob > F = 0.0013).

Cholesterol diet induced a decrease of both HDL and LDL cholesterol compared to the animals fed with the standard diet, and this decrease was detectable as early as week 8 (Figures 5A and 5C). Guinea pigs treated with both 3 and 30 mg/kg CRAC doses showed higher HDL cholesterol levels at week 14 compared to the untreated animals (Figure 5B) by 41% and 18%, respectively (F Ratio = 6.576, Prob > F = 0.0089). However, no effect of the treatment was observed on LDL cholesterol (Figure 5D).

Effect of CRAC Peptide on the Cholesterol-Induced Atheroma Formation in Guinea Pig Aortas

14 weeks of cholesterol-enriched diet induced the formation of important atheroma and lipid deposition in guinea pig aortas (Figures 6C and 6D) as compared

to animals fed with a standard diet (Figures 6A and 6B). Oil red staining revealed that the atheroma plaques formed contain an important quantity of lipids. The aorta seen in Figure 6 is almost completely obstructed. Both CRAC doses used, 3 (Figures 6E and 6F) and 30 mg/kg (Figures 6G and 6H), prevented the formation of atheroma deposits and no lipid-containing elements were visible in CRAC-treated guinea pigs.

Effect of CRAC Treatment on the Cholesterol-Induced Oxidative Stress in the Aorta Wall Revealed by Nitrotyrosine Immunostaining

Atheroma is associated with oxidative stress and protein nitrosylation resulting from the adduct formation between nitric oxide and tyrosines. The nitrotyrosine immunostaining was dramatically increased in the aorta of the cholesterol-enriched diet fed guinea pigs revealing the presence of an important oxidative stress (Figure 7B) as compared to the standard diet fed animals (Figure 7A). Treatment with either CRAC doses dramatically reduced the nitrotyrosine staining (Figures 7C and 7D) and brought it back to the levels seen in controls (Figure 7A), suggesting that the reduction of the atheroma formation (Figure 6) was associated with a decrease of the associated oxidative stress.

CRAC Peptide Treatment Reduced Cholesterol Diet-Induced Serum Creatine Kinase Level Increase

Serum creatine kinase has been used as a marker of cardiac and muscular suffering. Cholesterol diet induced a progressive and sustained increase of CK levels in guinea pig serum compared to the control. This increase was detectable at week 8 (1199 ±7 62 UI/L, $n = 15$, versus 216 ± 75 UI/L, $n = 22$, $p < 0.001$) and was even more pronounced at week 14 (3105 ± 1168 UI/L, $n = 7$, versus 212 ± 54 UI/L, $n = 3$, $p < 0.001$) (Figure 8A). Both 3 and 30 mg/kg CRAC doses dramatically reduced the CK increase observed in the serum of guinea pigs fed with the cholesterol-enriched diet by 65% and 60% respectively (F ratio = 5.945, Prob > F = 0.009). These results suggest that the heart and/or the muscular system of these animals are in a less critical suffering status than the untreated guinea pigs fed with the high cholesterol diet.

Bile Cholesterol Level

Use of the 2% cholesterol diet induced a 30% increase of the bile cholesterol content compared to the standard diet fed guinea pigs (Figure 9). This increase was completely abolished by the two doses of CRAC (3 and 30 mg/kg, $p < 0.001$). CRAC treatment had no effect on bile basal cholesterol content.

Discussion

Hypercholesterolemia is a major concern in western countries as a main etiology for atherosclerosis and CHD. Cholesterol lowering drugs are extensively used and in particular statins have been proven to be efficient in reducing the morbidity and mortality of these two high cholesterol related conditions. Although the statins overall safety profile is very good (McKenney et al., 2006), some concerns have been raised regarding the nature of the side-effects (Bays, 2006) essentially because they seem completely linked to the mechanism of action of this class of drugs. The most reported side-effect is myopathy (5%) and muscle pain that should be taken seriously since they are the first sign of a much more serious adverse event, namely rhabdomyolysis (Ambrosi et al., 2002) that led to cerivastatin market withdrawal in 2001. The inhibition of isoprenoids and Co-enzyme Q synthesis and consecutive impairment of the mitochondrial function as a consequence of the HMG-CoA reductase inhibition is highly suspected to be responsible for these serious side-effects. In addition, there is not enough feedback regarding the potential effect of the inhibition of the neuronal isoprenoids and Co-enzyme Q synthesis associated to the inhibition of brain cholesterol synthesis on the development of central neuropathy. Although these side-effects are fortunately rare, the application of the new recommendations for more aggressive treatment of hypercholesterolemia raises the question of their incidence in the near future.

In the spontaneously hypercholesterolemic ApoE knock-out B6.129P2-Apoetm1Unc/J mice, CRAC treatment reduced in a dose-dependent manner serum total cholesterol levels. It is noteworthy that this effect was obtained after only 14 days of treatment in an animal model that is genetically engineered to display very high cholesterol serum concentrations, thus mimicking in that aspect the conditions present in human familial hypercholesterolemia (Meir and Leitersdorf, 2004). CRAC treatment every other day for 7 weeks also reduced in a dose-dependent

manner the diet-induced hypercholesterolemia in guinea pigs. In these studies, it was observed that the individual cholesterol increase between week 8 and week 14 was 169% for the untreated group whereas it was 106% for the group that received the 3 mg/kg dose and only 34% for the animals treated with the highest dose of CRAC. It is interesting that this effect took place in animals continuously fed with the cholesterol-enriched diet, suggesting that in a standard therapeutic procedure where specific low cholesterol diets are prescribed, the CRAC effect could occur at lower doses. It is of major importance that guinea pigs treated with CRAC had higher HDL levels than control untreated animals fed with the high cholesterol diet. Indeed, HDL cholesterol serum concentrations were 41% and 18% higher than the control untreated guinea pigs. In comparison, a recent report showed that, at best, statins increase HDL in hypercholesterolemic human by 6% resulting in a 25-30% decrease of the probability to develop CHD (Rosenson, 2006). High cholesterol diet induced a dramatic decrease of LDL cholesterol which was an unexpected result. Interestingly, lowering LDL in the presence of 10-fold higher serum cholesterol levels did not prevent the formation of atheromas in the aorta of high cholesterol fed animals. CRAC treatment did not affect LDL cholesterol levels. Recent data suggest that increasing the concentrations of HDL cholesterol may be a more relevant therapeutic target than lowering LDL cholesterol in the prevention of CHD and atheroma formation (Lee and Choudhury, 2006; Rosenson, 2006; Toth et al., 2006). These data are supported by clinical data obtained with the newly developed drug torcetrapib, an inhibitor of cholesteryl ester transfer protein, that increases HDL cholesterol up to 73% in hypercholesterolemic patients (Clark et al., 2004). Taking these data into consideration, the CRAC peptide profile may represent a seducing therapeutic approach.

The total cholesterol lowering effect of CRAC was associated with a decrease in serum free cholesterol and a decrease of the bile total cholesterol. The main sources of free cholesterol in the serum are LDL cholesterol and chylomicron. Thus, a decrease of serum free cholesterol suggests that the free cholesterol carried by one or both of these sources is targeted by the CRAC peptide. The decrease of the bile cholesterol may be easily explained by the reduction of the free and total

circulating cholesterol. However, since the gallbladder is part of the cholesterol entero-hepatic circulation system, circulation could be affected by the CRAC treatment. Indeed, CRAC bound to cholesterol may be eliminated through the bile and the complex formed may prevent cholesterol reabsorption by the intestine identically to the bile acid sequestrants (Insull, 2006). Thus, since 50% to 75% of the cholesterol eliminated through the bile is reabsorbed by the intestine (Figure 10), it would explain, at least in part, the efficacy of CRAC at lowering the diet-induced hypercholesterolemia.

As expected, elevated cholesterol diet led to increased oxidative stress and the development of atheromas in the guinea pig aortas. Both CRAC doses used completely washed out the aortic deposits with the same efficacy and decreased the nitrotyrosine immunostaining back to what we observed in the standard diet fed animals, suggesting that CRAC treatment, even at the lowest dose used in this study, suppressed the atheroma-associated oxidative stress. The mechanism by which CRAC removes aortic atheromas remains to be determined but it is likely that the increase of HDL cholesterol as well as the ability of the peptide to bind cholesterol with high affinity (Li and Papadopoulos, 1998; Li et al., 2001; Jamin et al., 2005) suggest a direct action on the plaque where it removes cholesterol, thus contributing to the effect observed. Interestingly, the development of atheromas was associated with a dramatic increase in serum CK levels, suggesting that the heart of the exposed animals was under stress and suffering. Both CRAC doses used reduced the CK elevation by more than 60% in a manner that parallels the increase in HDL cholesterol and removal of the atheromas arguing in favor of a restoration of the coronary/heart function.

Atherosclerosis and CHD are two high cost burden pathologies for which many therapeutic alternatives to fibrates and statins remain unexplored. A novel treatment is described herein which employ an eight amino acid CRAC peptide found in the carboxy terminus of PBR, a high-affinity cholesterol and drug binding protein that transports and translocates cholesterol into the mitochondria of steroidogenic cells (Papadopoulos et al., 2006), a function conserved across species and kingdoms (Lindemann et al., 2004). The effect of the treatment in reducing

circulating cholesterol levels was validated in two hypercholesterolemia animal models, a genetically engineered mouse model mimicking familial hypercholesterolemia, and a diet-induced hypercholesterolemia. In addition, the CRAC peptide significantly increased HDL cholesterol, removed aortic formed atheroma and decreased the associated oxidative stress. Although the mechanism of action of CRAC may explain its ability to bind and alter the cholesterol-apolipoprotein interaction and remove atheromas, the data presented herein also suggest that CRAC might interfere with the cholesterol entero-hepatic circulation. Taken together all these data suggest that CRAC peptide might represent an interesting prototypical drug that could be used alone or in association with existing lipid lowering treatments.

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All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

WHAT IS CLAIMED IS:

1. A method to inhibit or treat hypercholesterolemia, comprising: administering to a mammal in need thereof, an effective amount of a composition comprising a cholesterol recognition/interaction amino acid consensus sequence comprising

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid.

2. A method to inhibit or treat hypercholesterolemia, comprising: administering to a mammal in need thereof an effective amount of a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

3. The method of claim 1 wherein Z is Leucine or Valine.

4. The method of claim 1 or 3 wherein B is Tyrosine.

5. The method of claim 1 or 3 wherein J is Arginine or Lysine.

6. The method of claim 1 wherein Z is Leucine or Valine, J is Arginine or Lysine, and B is Tyrosine.

7. The method of any one of claims 1 to 6 wherein X is 1 amino acid.

8. The method of any one of claims 1 to 6 wherein X is 2 amino acids.

9. The method of any one of claims 1 to 6 wherein X is 1 to 3 amino acids.

10. A method for reducing serum free cholesterol and bile total cholesterol in a mammal, comprising administering to a mammal in need thereof an effective amount of a composition comprising cholesterol recognition/interaction amino acid consensus sequence comprising

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid.

11. A method for reducing serum free cholesterol and bile total cholesterol in a mammal, comprising administering to a mammal in need thereof an effective amount of an isolated peptide having a cholesterol recognition/interaction amino acid sequence U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

12. The method of claim 1, 2, 10 or 11 wherein VLNYYVWR (SEQ ID NO:1) is administered.

13. A method for increasing HDL levels in a mammal, comprising administering to a mammal in need thereof an effective amount of a composition comprising cholesterol recognition/interaction amino acid consensus sequence comprising

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid.

14. A method for increasing HDL levels in a mammal, comprising administering to a mammal in need thereof an effective amount of a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid

residues, and wherein X is absent or 1 to 7 amino acid residues in an amount effective to increase HDL levels.

15. The method of claim 1, 2, 10, 11, 13 or 14 wherein the composition comprises about 3 mg/kg to 30 mg/kg of the consensus sequence or peptide.

16. The method of claim 13 or 14 wherein HDL levels are increased by at least 10%.

17. A method to inhibit or treat viral infection, comprising:
administering to a mammal in need thereof, an effective amount of a composition comprising a cholesterol recognition/interaction amino acid consensus sequence comprising

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid.

18. A method to inhibit or treat viral infection, comprising:
administering to a mammal in need thereof an effective amount of a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

19. A method to inhibit or treat obesity, comprising:
administering to a mammal in need thereof, an effective amount of a composition comprising a cholesterol recognition/interaction amino acid consensus sequence comprising

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid.

20. A method to inhibit or treat obesity, comprising:
administering to a mammal in need thereof an effective amount of a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

21. A method to inhibit or treat diabetes or glucose intolerance, comprising:
administering to a mammal in need thereof, an effective amount of a composition comprising a cholesterol recognition/interaction amino acid consensus sequence comprising

$$Z\text{-}(X)_{1\text{-}5}\text{-}B\text{-}(X)_{1\text{-}5}\text{-}J$$

wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid.

22. A method to inhibit or treat diabetes or glucose intolerance, comprising:
administering to a mammal in need thereof an effective amount of a composition comprising an isolated peptide having a cholesterol recognition/interaction amino acid sequence U-L-N-Y-X, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

23. The method of claim 1, 10, 13, 17, 19 or 21 wherein the composition comprises a molecule having the cholesterol recognition/interaction amino acid consensus sequence.

24. The method of claim 23 wherein the molecule has a molecular weight of less than about 5,000.

25. The method of claim 23 wherein the molecule has a molecular weight of less than about 2,000.

26. The method of claim 2, 11, 14, 18, 20 or 22 wherein the peptide has 50 residues or less.

27. The method of claim 2, 11, 14, 18, 20 or 22 wherein the peptide has 25 residues or less.

28. The method of claim 2, 11, 14, 18, 20 or 22 wherein the peptide has 20 residues or less.

29. Use of a molecule having a cholesterol recognition/interaction amino acid consensus sequence comprising $Z-(X)_{1-5}-B-(X)_{1-5}-J$ in the manufacture of a medicament to inhibit or treat disorders associated with aberrant cholesterol levels in a mammal, wherein Z is a neutral hydrophobic amino acid, B is a neutral polar amino acid, J is a basic amino acid and X is any amino acid.

30. Use of an isolated peptide having a cholesterol recognition/interaction amino acid sequence U-L-N-Y-X in the manufacture of a medicament to inhibit or treat disorders associated with aberrant cholesterol levels in a mammal, wherein U is absent or 1 to 6 amino acid residues, and wherein X is absent or 1 to 7 amino acid residues.

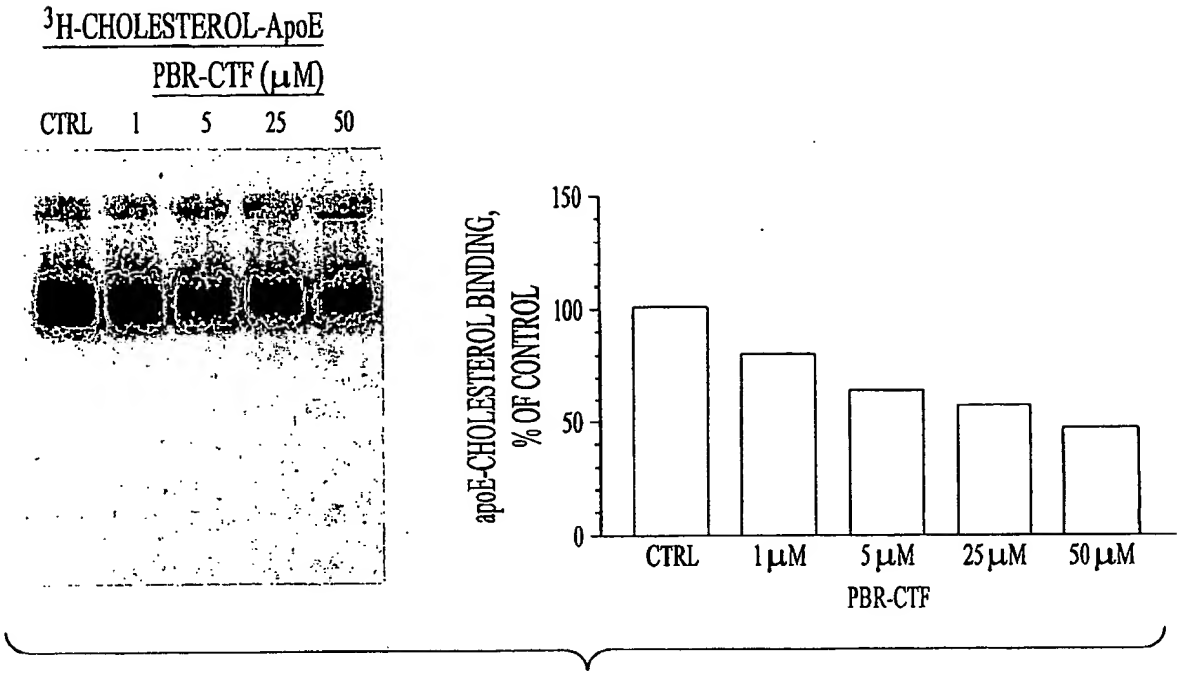

3H-CHOLESTEROL-ApoE
PBR-CTF (μM)
CTRL 1 5 25 50
apoE-CHOLESTEROL BINDING, % OF CONTROL
150
100
50
0
CTRL
1 μM
5 μM
25 μM
50 μM
PBR-CTF

FIG. 1A

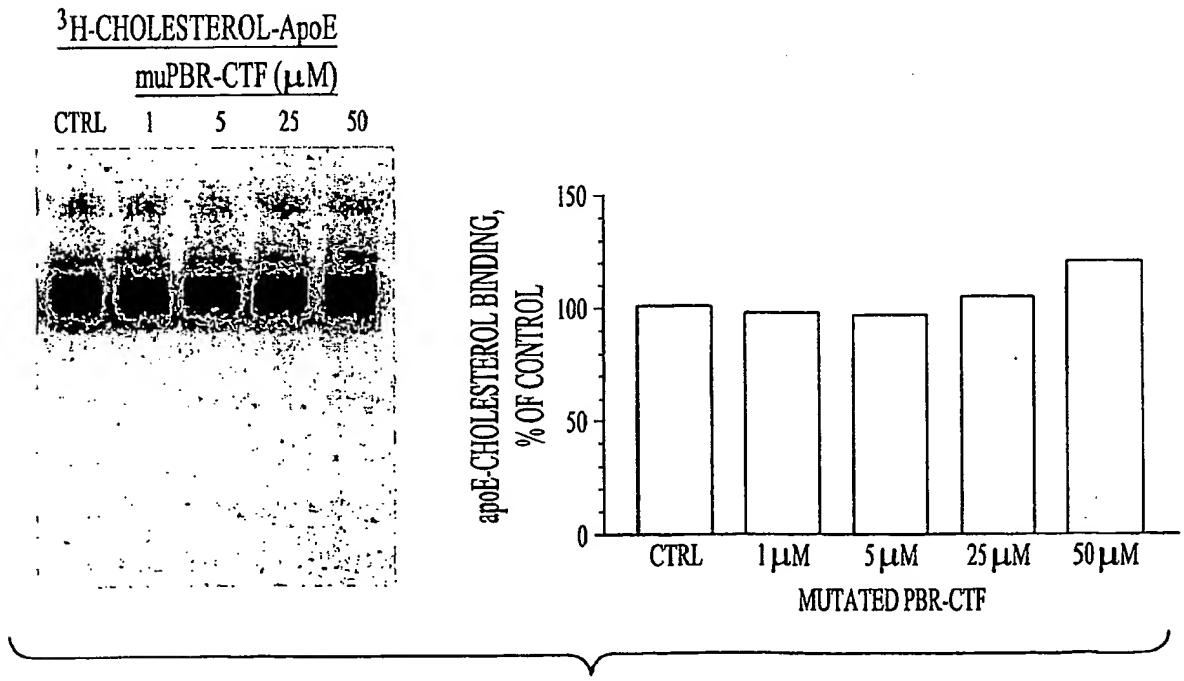

3H-CHOLESTEROL-ApoE
muPBR-CTF (μM)
CTRL 1 5 25 50
apoE-CHOLESTEROL BINDING, % OF CONTROL
150
100
50
0
CTRL
1 μM
5 μM
25 μM
50 μM
MUTATED PBR-CTF

FIG. 1B

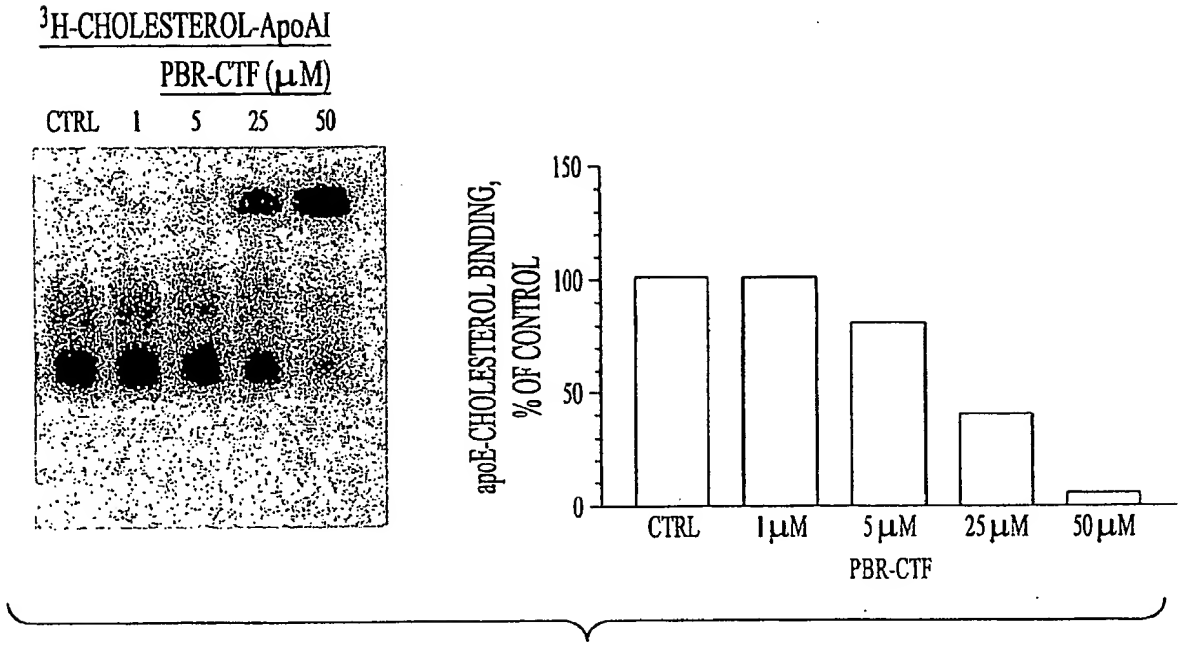

3H-CHOLESTEROL-ApoAI
PBR-CTF (μM)
CTRL 1 5 25 50
apoE-CHOLESTEROL BINDING, % OF CONTROL
150
100
50
0
CTRL
1μM
5μM
25μM
50μM
PBR-CTF

FIG. 1C

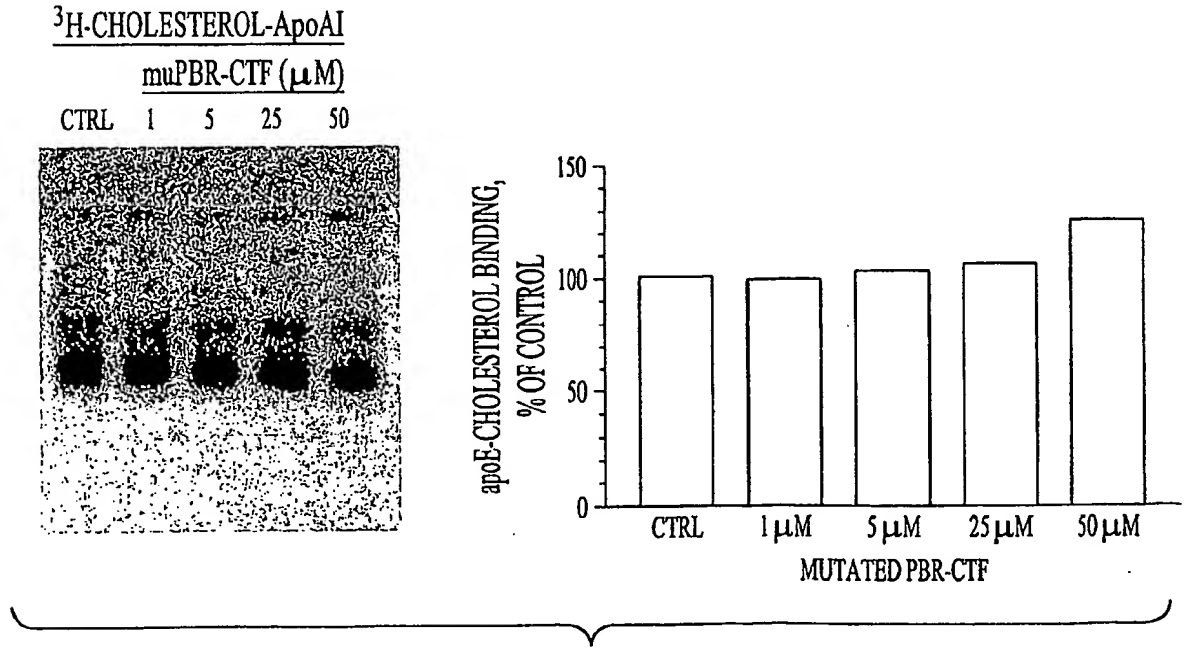

3H-CHOLESTEROL-ApoAI
muPBR-CTF (μM)
CTRL 1 5 25 50
apoE-CHOLESTEROL BINDING, % OF CONTROL
150
100
50
0
CTRL
1μM
5μM
25μM
50μM
MUTATED PBR-CTF

FIG. 1D

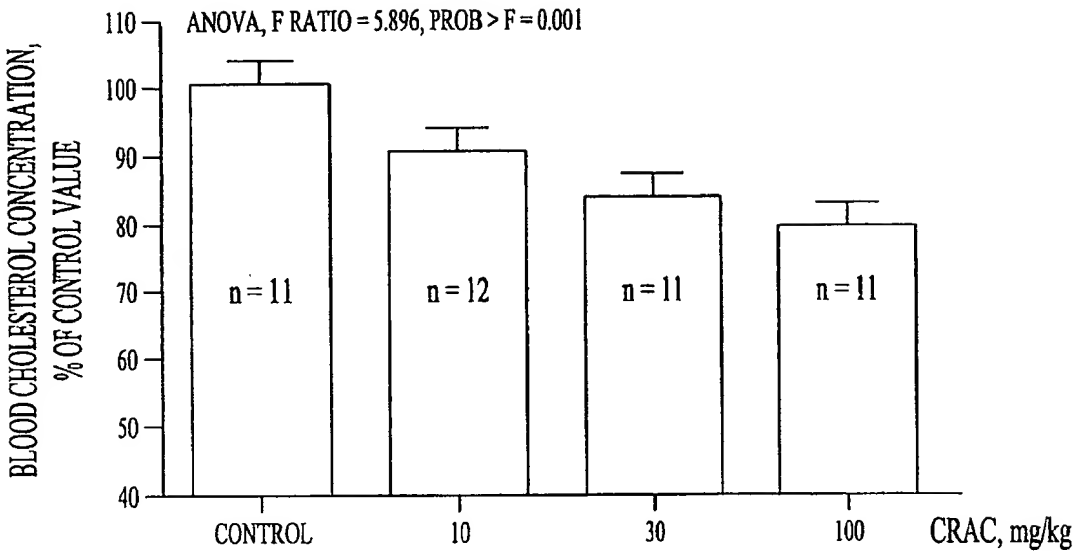

ANOVA, F RATIO = 5.896, PROB > F = 0.001
BLOOD CHOLESTEROL CONCENTRATION, % OF CONTROL VALUE
110
100
90
80
70
60
50
40
n = 11
n = 12
n = 11
n = 11
CONTROL
10
30
100
CRAC, mg/kg

FIG. 2

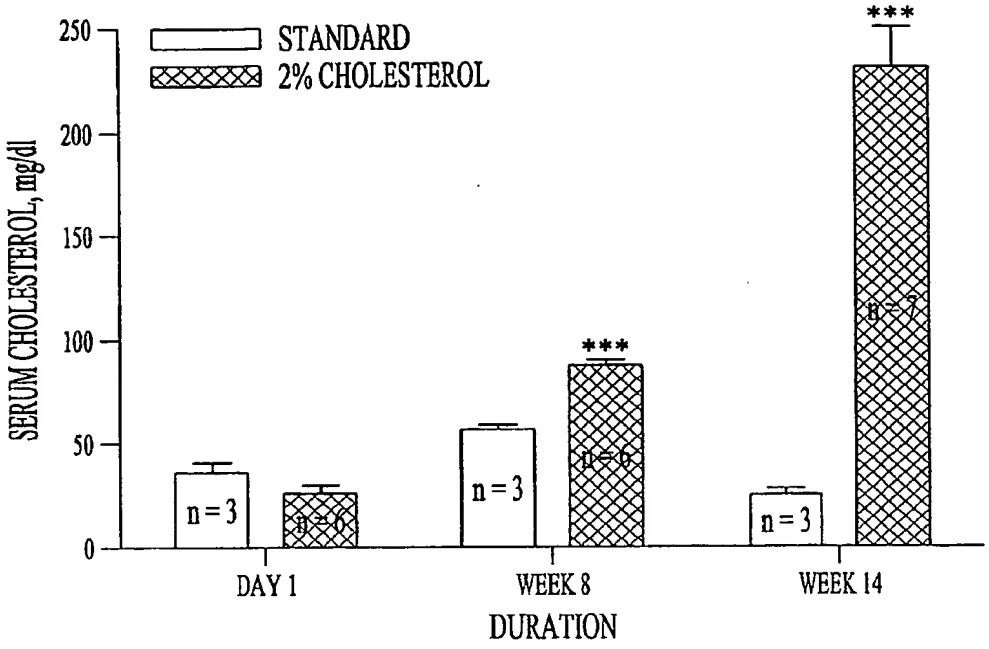

STANDARD
2% CHOLESTEROL
SERUM CHOLESTEROL, mg/dl
250
200
150
100
50
0
n = 3
n = 6
n = 3
n = 6
n = 3
n = 7
***
***
DAY 1
WEEK 8
WEEK 14
DURATION

FIG. 3A

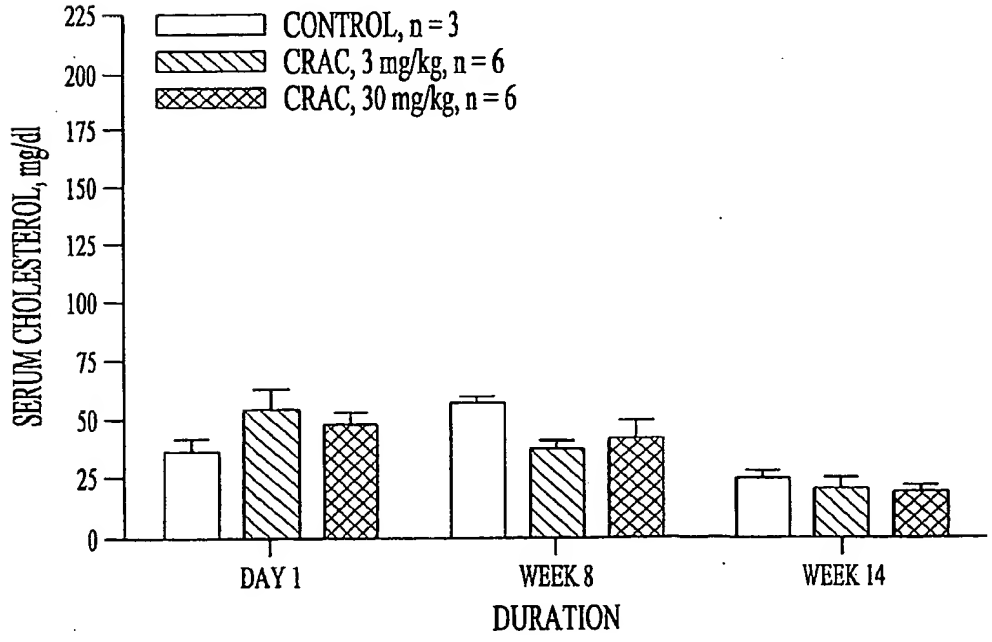

CONTROL, n = 3
CRAC, 3 mg/kg, n = 6
CRAC, 30 mg/kg, n = 6
SERUM CHOLESTEROL, mg/dl
225
200
175
150
125
100
75
50
25
0
DAY 1
WEEK 8
WEEK 14
DURATION

FIG. 3B

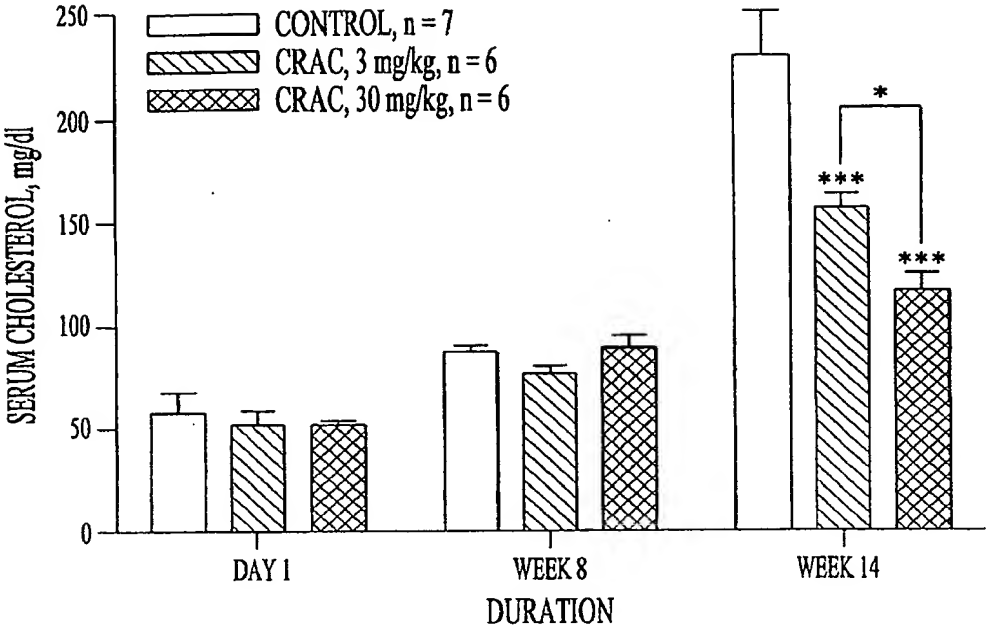

CONTROL, n = 7
CRAC, 3 mg/kg, n = 6
CRAC, 30 mg/kg, n = 6
SERUM CHOLESTEROL, mg/dl
250
200
150
100
50
0
*
***
***
DAY 1
WEEK 8
WEEK 14
DURATION

FIG. 3C

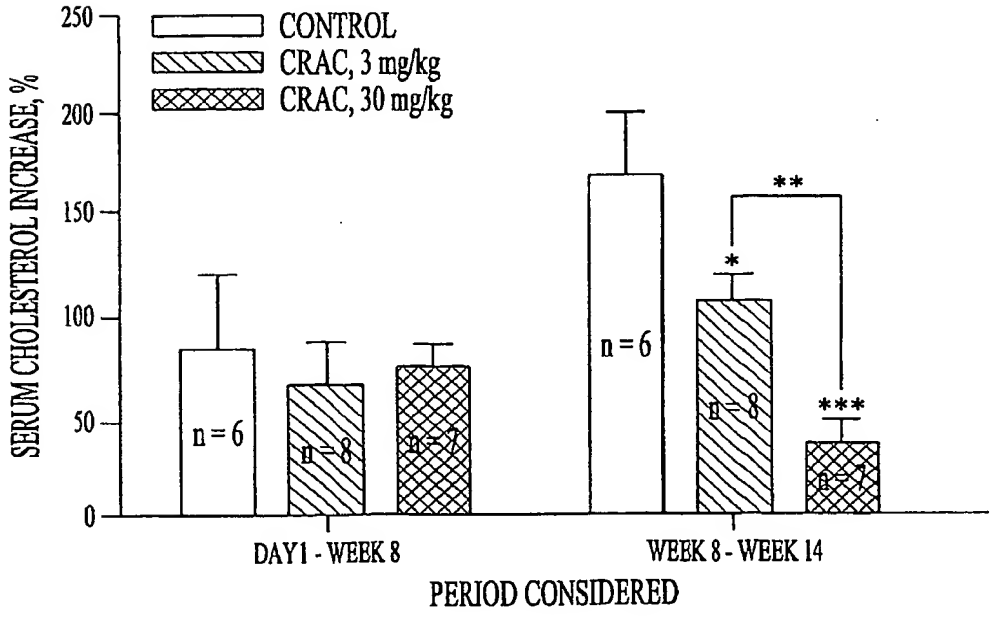

CONTROL
CRAC, 3 mg/kg
CRAC, 30 mg/kg
SERUM CHOLESTEROL INCREASE, %
250
200
150
100
50
0
n = 6
n = 8
n = 7
n = 6
n = 8
n = 7
**
*
***
DAY1 - WEEK 8
WEEK 8 - WEEK 14
PERIOD CONSIDERED

FIG. 3D

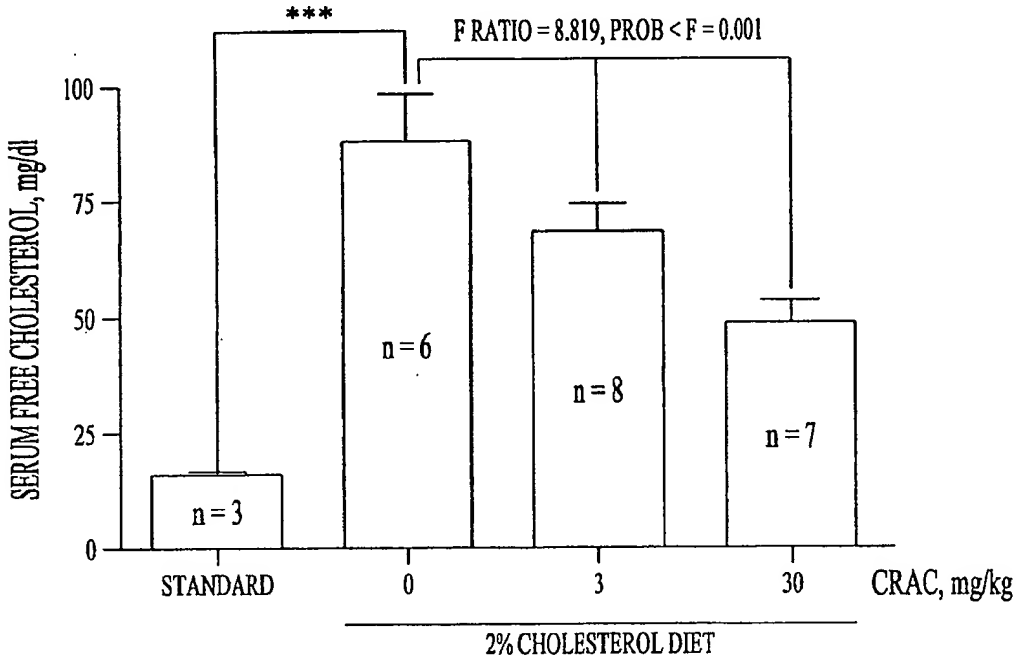

***
F RATIO = 8.819, PROB < F = 0.001
SERUM FREE CHOLESTEROL, mg/dl
100
75
50
25
0
n = 3
n = 6
n = 8
n = 7
STANDARD
0
3
30
CRAC, mg/kg
2% CHOLESTEROL DIET

FIG. 4

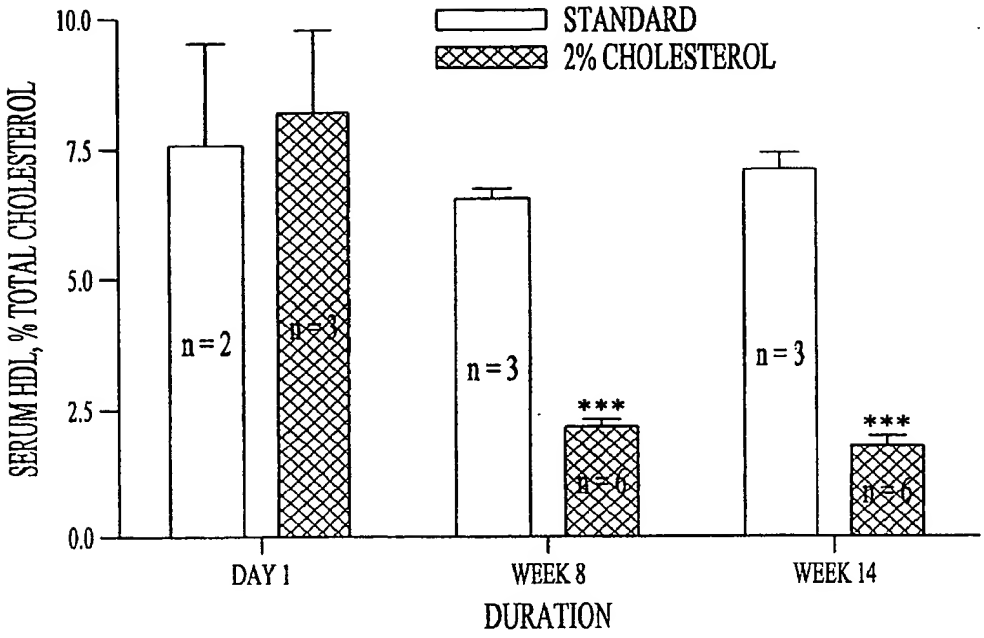

STANDARD
2% CHOLESTEROL
SERUM HDL, % TOTAL CHOLESTEROL
10.0
7.5
5.0
2.5
0.0
n = 2
n = 3
n = 3
n = 6
n = 3
n = 6
***
***
DAY 1
WEEK 8
WEEK 14
DURATION

FIG. 5A

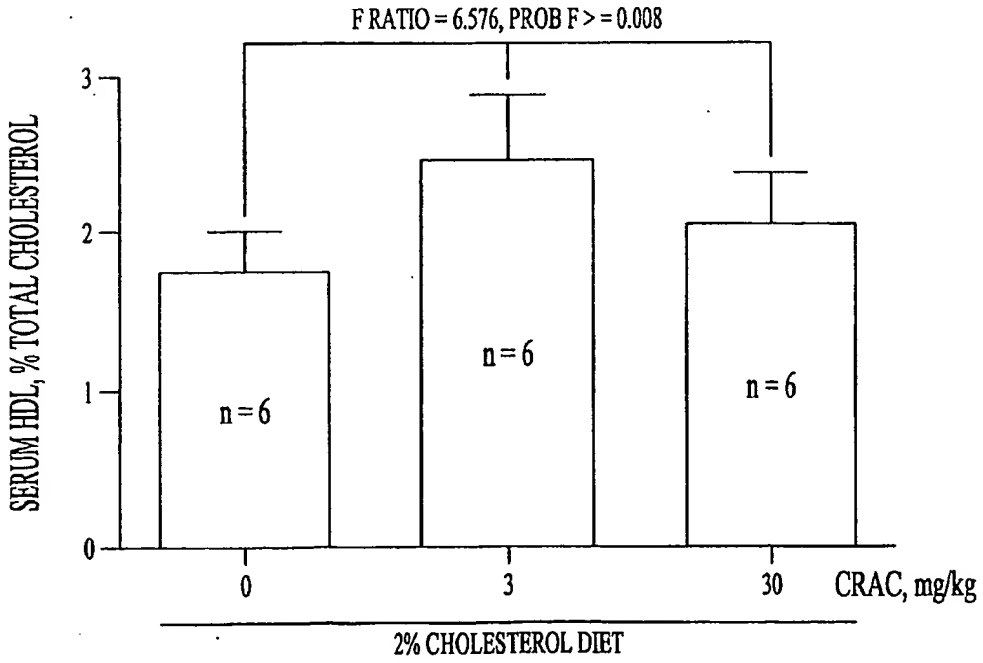

F RATIO = 6.576, PROB F > = 0.008
SERUM HDL, % TOTAL CHOLESTEROL
3
2
1
0
n = 6
n = 6
n = 6
0
3
30
CRAC, mg/kg
2% CHOLESTEROL DIET

FIG. 5B

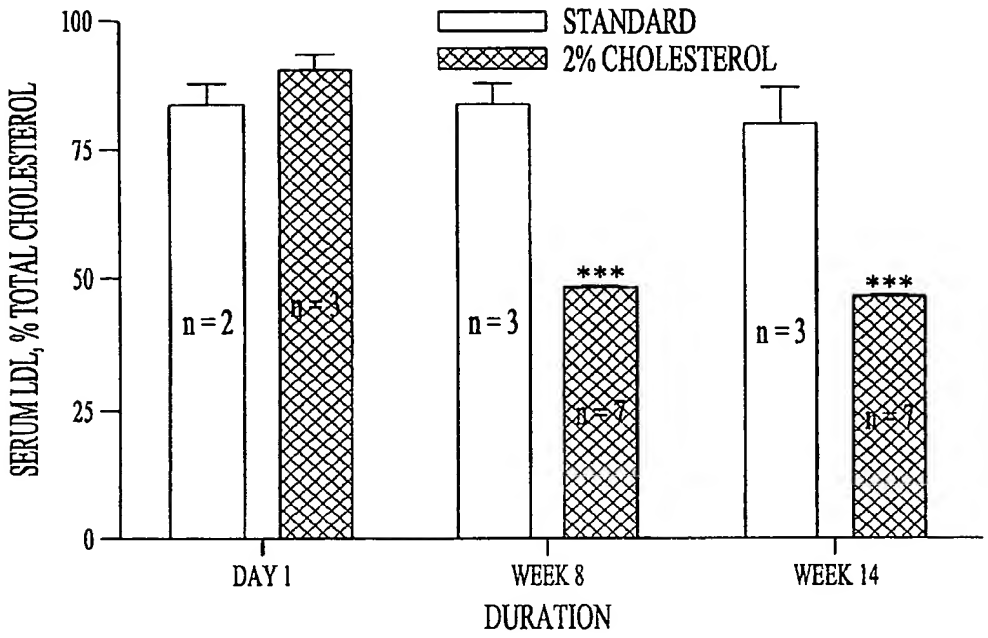

STANDARD
2% CHOLESTEROL
SERUM LDL, % TOTAL CHOLESTEROL
100
75
50
25
0
n = 2
n = 3
n = 3
n = 7
n = 3
n = 7
***
***
DAY 1
WEEK 8
WEEK 14
DURATION

FIG. 5C

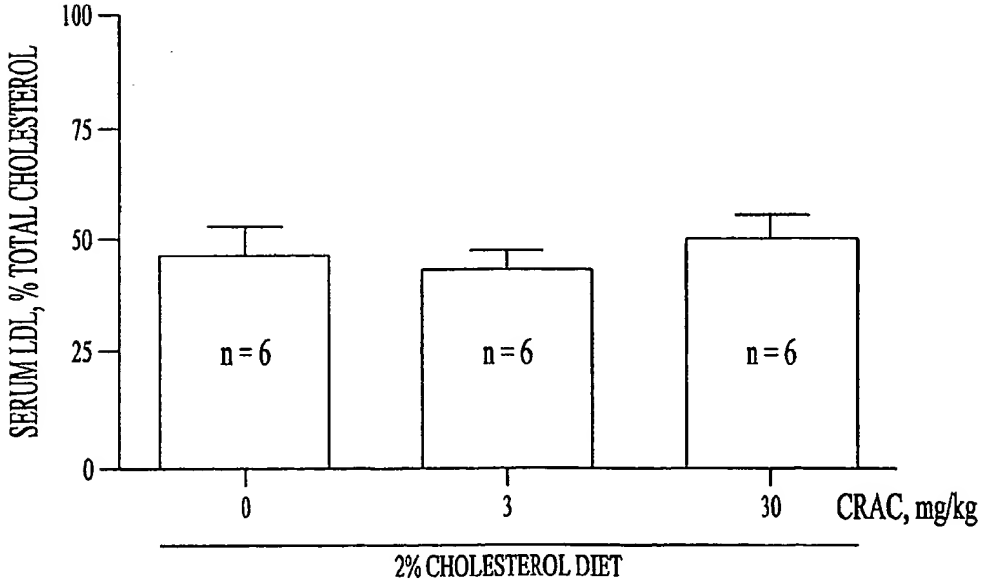
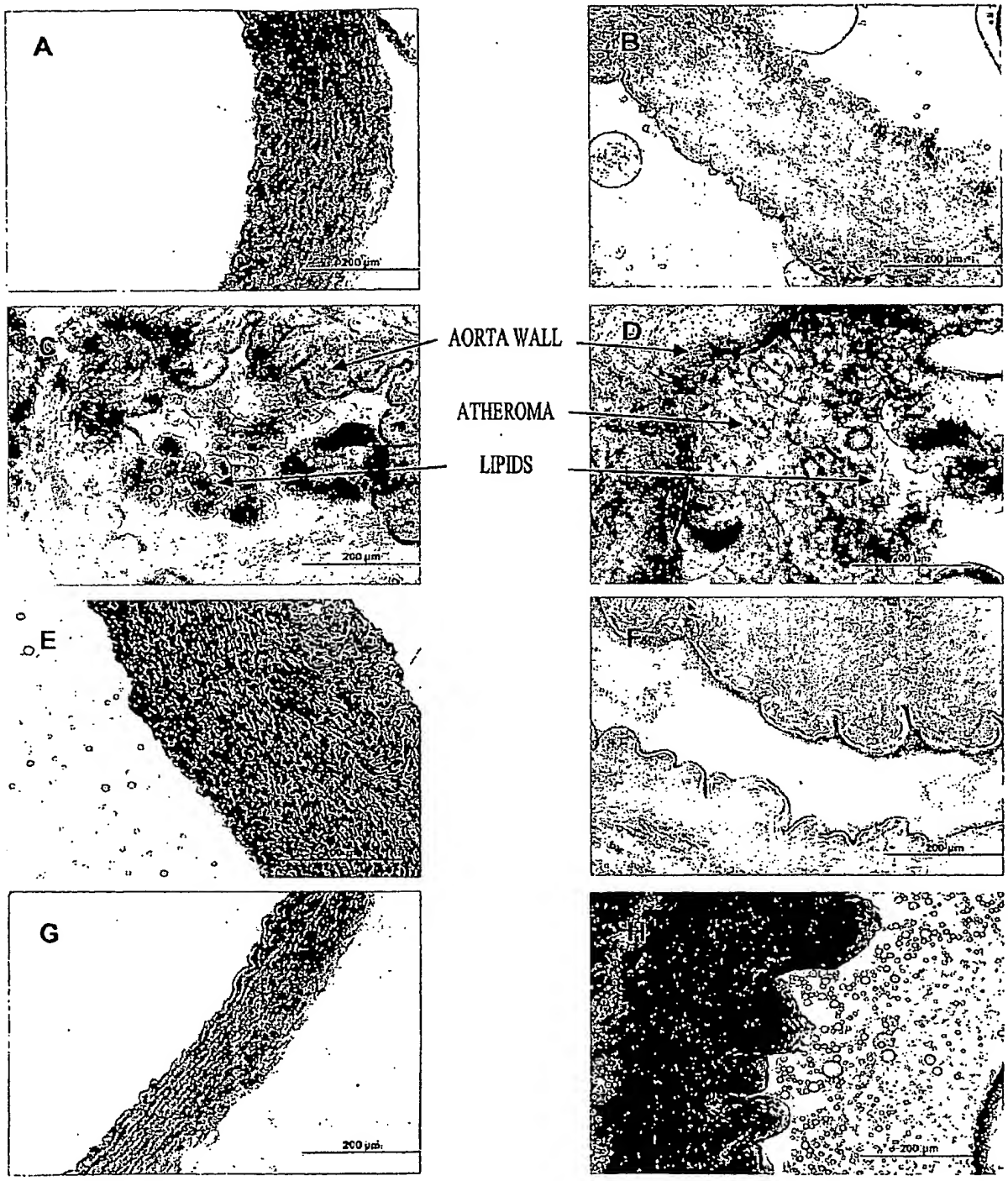
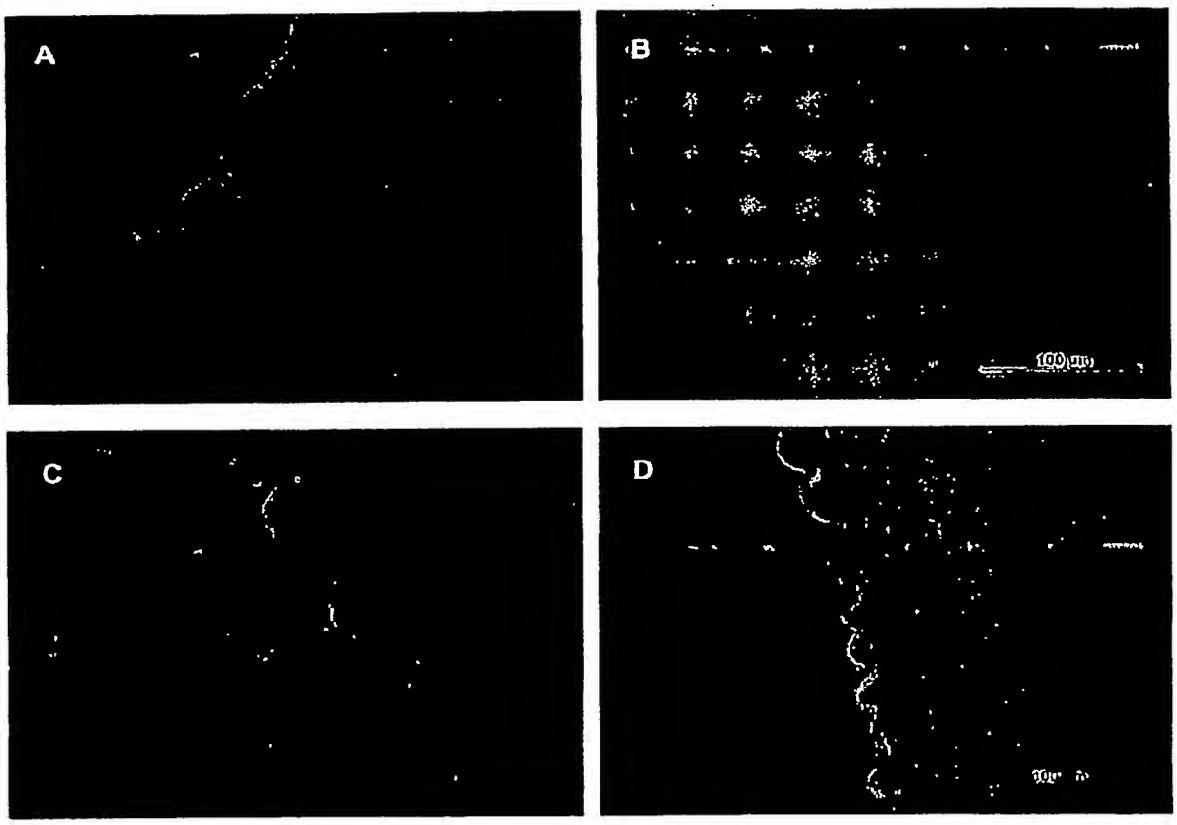

SERUM LDL, % TOTAL CHOLESTEROL
100
75
50
25
0
n = 6
n = 6
n = 6
0
3
30
CRAC, mg/kg
2% CHOLESTEROL DIET

FIG. 5D



A AND B: STANDARD DIET
C AND D: 2% CHOLESTEROL DIET
E AND F: 2% CHOLESTEROL DIET + CRAC 3 mg/kg
G AND H: 2% CHOLESTEROL DIET + CRAC 30 mg/kg

FIG. 6



A: STANDARD DIET
B: 2% CHOLESTEROL DIET
C: 2% CHOLESTEROL DIET + CRAC 3 mg/kg
D: 2% CHOLESTEROL DIET + CRAC 30 mg/kg

FIG. 7

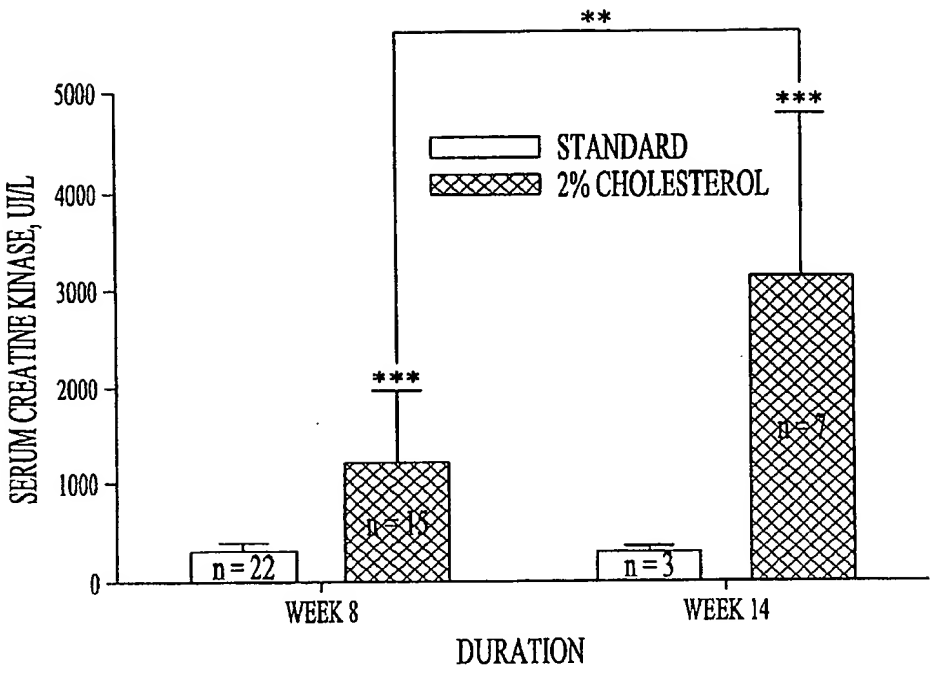

**
***
***
STANDARD
2% CHOLESTEROL
SERUM CREATINE KINASE, UI/L
5000
4000
3000
2000
1000
0
n = 22
n = 13
n = 3
n = 7
WEEK 8
WEEK 14
DURATION

FIG. 8A

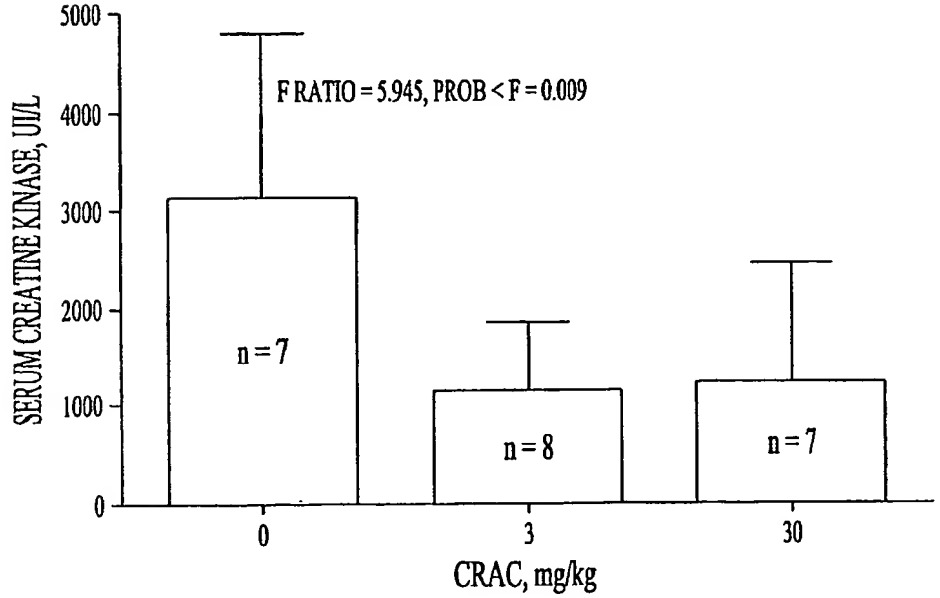

F RATIO = 5.945, PROB < F = 0.009
SERUM CREATINE KINASE, UI/L
5000
4000
3000
2000
1000
0
n = 7
n = 8
n = 7
0
3
30
CRAC, mg/kg

FIG. 8B

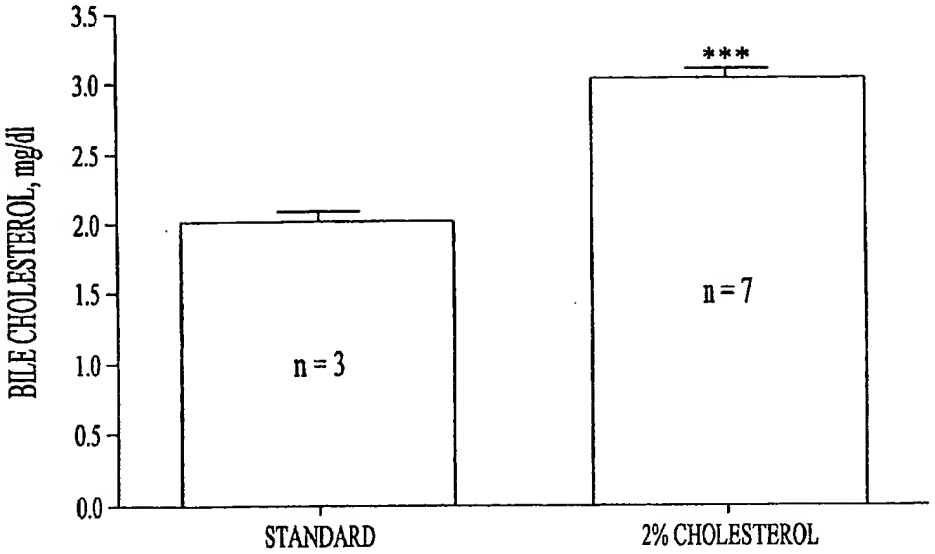

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
BILE CHOLESTEROL, mg/dl
***
n = 3
n = 7
STANDARD
2% CHOLESTEROL

FIG. 9A

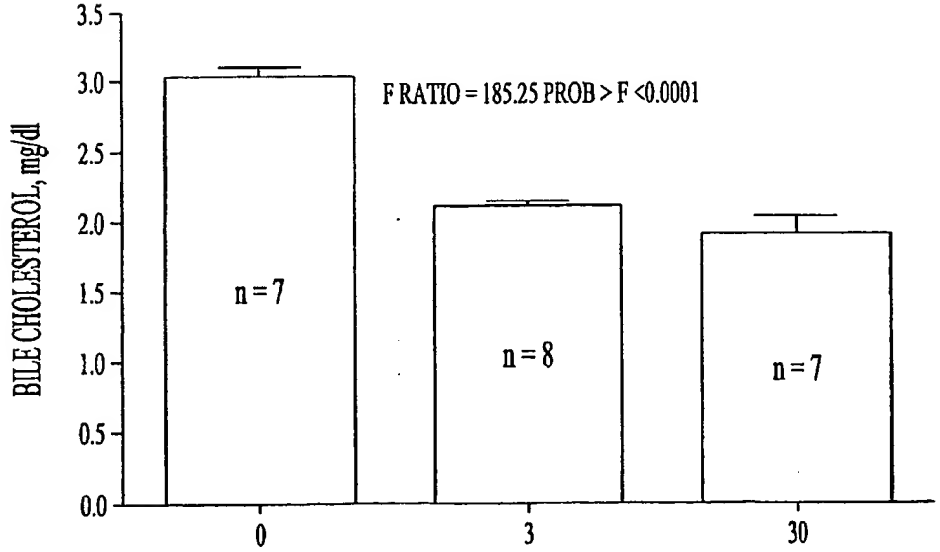

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
BILE CHOLESTEROL, mg/dl
F RATIO = 185.25 PROB > F <0.0001
n = 7
n = 8
n = 7
0
3
30

FIG. 9B

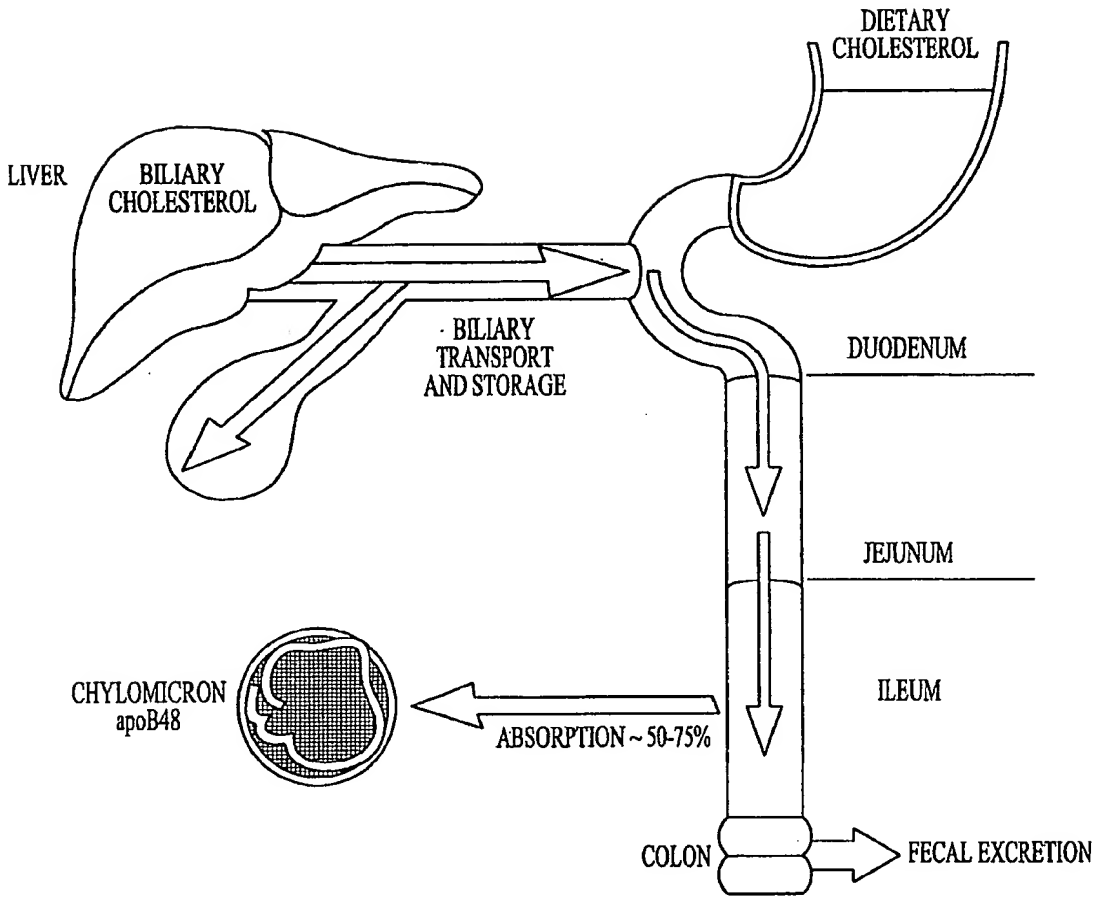

DIETARY
CHOLESTEROL
LIVER
BILIARY
CHOLESTEROL
BILIARY
TRANSPORT
AND STORAGE
DUODENUM
JEJUNUM
ILEUM
CHYLOMICRON
apoB48
ABSORPTION ~ 50-75%
COLON
FECAL EXCRETION

FIG. 10